STUDY ON QUANTITATIVE ASSESSMENT OF INSULIN RESPONSE IN IMPAIRED GLUCOSE REGULATION

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(PHARMACEUTICAL BIOTECHNOLOGY)

Submitted by
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ABBREVIATIONS

1/2II	-	Half an hour insulinogenic index.
AIR	-	Acute insulin response
AUC	-	Area under the curve
AUCg	-	Area under the curve glucose.
BMI	-	Body mass index
FSIVGTT	-	Frequently sampled intravenous glucose tolerance test
GIP	-	Glucose Dependent Insulino Tropic
GLP	-	Glucogan Like Peptide
HEC	-	Hyper insulinemic euglycemic clamp
HGP	-	Hepatic Glucose Production
HOMA	-	Homeostasis model assessment
IFG	-	Impaired fasting glucose
IGR	-	Impaired glucose regulation.
IGT	-	Impaired glucose tolerance.
IR	-	Insulin resistance
ISI	-	Insulin sensitivity index.
IST	-	Insulin suppression test.
IVGTT	-	Intravenous glucose tolerance test.

MINMOD	-	Minimal model analysis of frequently sampled intravenous glucose tolerance test
MODY	-	Maturity onset diabetes of the young.
NGR	-	Normal glucose regulation
NGT	-	Normal glucose tolerance.
OGTT	-	Oral glucose tolerance test.
		Polypeptide
QUICKI	-	Quantitative insulin sensitivity check index
SI	-	Index of Insulin sensitivity.
SSPG	-	Steady state plasma glucose
SSPI	-	Steady state plasma insulin

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OBJECTIVE OF THE STUDY

To examine metabolic abnormalities that characterizes pre-diabetes types for identifying more pre-diabetic subjects before they fall into the criteria of WHO & ADA and providing insight into development of therapeutic strategies to slow/halt their progression into type 2 diabetes.

- To model and extract metabolic information harbored by the shape of the blood glucose curve during an OGTT (along with the level of glycemia).
- To identify a reliable yet simple indirect method for quantitative assessment of insulin release and insulin sensitivity.
- To put forward cutoff values for different time points of OGTT & AUCg, to help diagnose IGR and diabetes.
- To examine whether a composite measure (oral disposition index) is associated with the development of diabetes.
- To examine the hyperbolic relationship between the early insulin release and surrogate measures of insulin sensitivity for identifying different glucose tolerance categories.

PURPOSE OF STUDY

India has the highest prevalence of diabetes accounting for nearly one-sixth of the world's diabetic patients. The risk factors peculiar for developing diabetes among Indians include high familial aggregation, central obesity, insulin resistance and life style changes due to urbanization. Most long standing macro and micro vascular complications are also more common among Indian diabetics as compared to other races and ethnic groups. The rising incidence of diabetes and its complications are going to pose a grave health care burden on our country.

Type 2 diabetes frequently goes undiagnosed for many years, because hyperglycemia develops gradually. Early detection, diagnosis, and early treatment of diabetes are very important for preventing diabetic complications. Clinical trials have demonstrated that lifestyle intervention and pharmacological therapy in high-risk individuals reduce the incidence of type 2 diabetes. Thus, reliable models for identification of individuals at high risk for future type 2 diabetes are essential and have important clinical implications for intervention programs.

The quantitative assessment of insulin resistance or insulin sensitivity is of great importance in the study of epidemiology and

pathophysiology of major public health problems and in following the clinical course of patients on various therapeutic regimens.

Ability to easily assess insulin sensitivity would be useful for investigating the role of insulin resistance in the pathophysiology of type 2 diabetes mellitus, polycystic ovary disease and many metabolic disturbances associated with coronary artery disease, including obesity, dyslipidemia, and hypertension. Therefore, there is a need for an accurate, reproducible and simple method for measuring insulin resistance.

INTRODUCTION

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long–term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non–ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death.

A variety of causes can manifest as diabetes mellitus. While most patients have Type 1 or Type 2 diabetes, other types occur. Identifying the cause is important for management and prognosis (Alberti KGMM ,*et al.* 1998).

Table 1: Classification of diabetes mellitus

Type of Diabetes	Features
Type 1	Absolute insulin deficiency following destruction of the beta-cells
Type 2	Relative insulin deficiency due to a combination of insulin resistance and impaired insulin secretion
Gestational	Diabetes triggered by pregnancy and often remitting afterwards
Other	Genetic or acquired

Other causes of diabetes or impaired glucose handling due to genetic cause primarily affecting insulin production or action:

(1) Genetic defect in beta-cell function

a. NeuroD1 and chromosome 2 (MODY 6)
b. Glucokinase and chromosome 7p (MODY 2)
c. HNF-1alpha and chromosome 12 (MODY 3)
d. Insulin promotor factor 1 and chromosome 13 (MODY 4)
e. HNF-1beta and chromosome 17 (MODY 5)
f. HNF-4alpha and chromosome 20q (MODY 1)
g. Mitochondrial DNA

(2) Genetic defect in insulin action (defect in insulin receptor, etc)

a. Type A insulin resistance
b. Leprechaunism
c. Lipoatrophic diabetes
d. Rabson-Mendenhall syndrome)

Other causes of diabetes or impaired glucose handling due to acquired causes:

(1) Disease of the exocrine pancreas

a. Pancreatitis
b. Cystic fibrosis
c. Hemochromatosis
d. Fibrocalculous pancreatopathy
e. Pancreatic tumor
f. Trauma or surgery

(2) Drug or chemical-induced

a. Corticosteroids
b. Treatment of aids
c. Organ transplantation (tacrolimus, other)
d. Thiazides
e. Interferon
f. Vacor
g. Nicotinic acid
h. Pentamidine
i. Second-generation anti-psychotic agents
j. Diazoxide
k. Dilantin
l. Beta-adrenergic agonists
m. Others

(3) Endocrinopathies

a. Acromegaly
b. Glucagonoma or somatostatinoma
c. Hyperthyroidism
d. Aldosternoma or Cushing's disease
e. Pheochromocytoma

(4) Viral infection-related

a. Cytomegalovirus (CMV)
b. Congenital rubella

(5) Immunologic disorders

a. Antibodies to insulin receptor
b. Stiff man syndrome

(6) Hereditary disorder associated diabetes (may include one or more of the above mechanisms)

a. Klinefelter's syndrome
b. Turner's syndrome
c. Friedrich's ataxia
d. Wolfram's syndrome
e. Prader-Willi syndrome
f. Down's syndrome
g. Myotonic dystrophy
h. Lawrence-Moon-Bardet-Biedl syndrome
i. Congenital porphyria
j. Others

Insulin –glucose dynamics: Glucose tolerance is an expression of the efficiency with which homeostatic mechanism restore glycemia to basal levels after perturbation. Clinically, the most assessment is following an oral glucose load ,a surrogate for a more physiological meal. The homeostatic response includes an increase in insulin levels and, therefore, also the insulin dependent processes that lower glycemia. Theoretically the oral glucose tolerance test should yield an estimate of insulin sensitivity, if insulin concentrations are measured. Glucose concentrations also change in manner that is partly dependent on insulin, but also on gastric emptying and absorption. In general, therefore attempts have been made to isolate the glucose –insulin relationship, as much as possible, from other factors (Katz A, *et al.* 2000).

Insulin is an essential peptide hormone whose metabolic actions maintain whole body glucose homeostasis and promote efficient glucose utilization (Accili D, *et al.* 2003). Insulin stimulates increased glucose disposal in skeletal muscle and adipose tissue, whereas it inhibits gluconeogenesis in liver to help regulate glucose homeostasis. In addition to these classical insulin target tissues, there are many other important physiological targets of insulin, including the brain, pancreatic ß-cells, heart, and vascular endothelium, that help to coordinate and couple metabolic and cardiovascular homeostasis under healthy conditions (Prodi E, *et al.* 2006). Insulin has concentration-dependent saturable actions to increase whole bodyglucose disposal. The maximal effect of insulin defines "insulin responsiveness," whereas the insulin concentration required for a half-maximal response defines "insulin sensitivity".

Insulin sensitivity and insulin secretion are mutually related such that insulin resistance is compensated by increased insulin secretion. A correct judgement of insulin secretion therefore requires validation in relation to the insulin sensitivity in the same subject.

INSULIN RESISTANCE

Insulin resistance/sensitivity is typically defined as decreased sensitivity or responsiveness to metabolic actions of insulin, such as insulin mediated glucose disposal and inhibition of hepatic glucose production(HGP). The concept of insulin resistance was proposed as early as (Himsworth H,*et al.* 1936) to describe diabetic patients

requiring high doses of insulin. insulin resistance plays a major pathophysiological role in type2 diabetes and is tightly associated major public problems, including obesity, hypertension, coronaryartery disease ,dyslipedemias ,andcardiovascular abnormalities that define the metabolic syndrome.(Reaven GM, *et al.* 2005,Petersen KF, *et al.* 2007).A global epidemic of obesity is driving the increased incidence and prevalence of type 2 diabetes and its cardiovascular complication(Giles TD, *et al.* 2007).

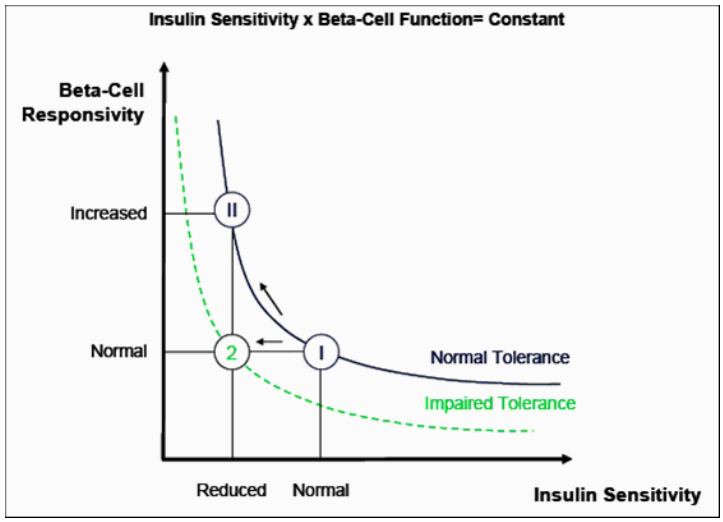
Insulin sensitivity vs insulin secretion

Insulin sensitivity vs insulin secretion – the hyperbolic relationship

It had already been shown several decades ago that insulin resistance such as in obesity is associated with an increased insulin secretion . Nevertheless, the close and inverse relationship between insulin secretion and insulin sensitivity has been widely acknowledged only during recent years. An early attempt at finding a mathematical relationship between insulin sensitivity and insulin secretion as defined by the pancreatic sensitivity to glucose.

Fig1: Insulin sensitivity and insulin secretion

an hyperbolic relationship



In recent years different mathematical formulas for measuring insulin resistance have been developed .(Hosker JP *et al.* 1985).these mathematical formulas use serum glucose and insulin levels(Quon MJ *et al.* 2001) either when patients are fasting or during oral glucose tolerance test(OGTT),which is considered to be a good physiological initiator of meal stimulation (Cederholm J *et al.* 1990).

SIMPLE SURROGATE INDEXES FOR INSULIN SENSITIVITY/RESISTANCE

In healthy humans, the fasting condition represents a basal steady state where glucose is homeostatically maintained in the normal range such that insulin levels are not significantly changing and HGP is constant; i.e, basal insulin secretion by pancreatic –β cells determines a relatively constant level of insulinemia that will be lower or higher in accordance with insulin sensitivity/resistance

such that HGP matches whole body glucose disposal under fasting conditions.

A critical condition and assumption of simple surrogate indexes is that subjects are strictly fasting and in a basal steady-state condition with respect to glycemia, insulinemia, and HGP. Surrogate indexes based on fasting glucose and insulin concentrations reflect primarily hepatic insulin sensitivity/resistance. However, undermost conditions, hepatic and skeletal muscle insulin sensitivity/resistance are proportional to each other. Therefore, definitions of the more useful surrogate indexes take these considerations into account. Due to lack of a standardized insulin assay, it is not possible to use surrogate indexes to define universal cutoff points for insulin resistance.

General advantages and appropriate usage:

Simple surrogate indexes of insulin sensitivity/resistance are inexpensive quantitative tools that can be easily applied in almost every setting, including epidemiological studies, large clinical trials, clinical research investigations, and clinical practice.

Table 2: Insulin Sensitivity Indices

Index	Equation
INS^{-1}	$1/INS_0$
GLUCOSE TO INSULIN RATIO	$GLU0/INS_0$
HOMA IR	$I_0G_0/22.5$
RAYNAUD	$40/INS_0$
BELFOIRE(F)	$2\ I_0G_0+1$
$FIRI^{-1}$	$1/\ I_0*\ G_0/22.5$
QUICKI	$1/LOG\ I_0\ /LOG\ G_0$
$INS120^{-1}$	1/1NS120
GLUCOSE INSULIN AUC	AUCGLU/AUCINS
MCAULEY	$Mffm/I=e^{(2.63-0.2*ln(I0)-0.31\ ln\ (TAG0)}$
CEDERHOLM	$\frac{75000+(G-2HG)*(1.15*180)*0.19*m)}{120*\ Gmean\ *log\ (I\ mean)}$
BELFOIRE(OG)	2/IAUC*GAUC+1
DRIVSHOLM	GLU AUC/INS AUC
GUTT	[75.000+(fasglu-2hglu)*0.19*bw]/120min
ISI MATSUDA	$\frac{10000}{\sqrt{GLU*INS*GLUMEAN*INSMEAN}}$
SOONTHURNPUN	$\frac{[1.9/6*BW*G+5201.9/18*BW*GAUC*GU/1.8]*1000}{(IAUC*BW)}$
AVIG NON SiB Si2h VD	[(0.317*SiB)+Si2h]/2 108/I0*G0*VD 108/I120*G120*VD 150*BW

INSULIN SENSITIVITY INDICES

INS^{-1} -1/INS0 (1/ FASTING INSULIN): In healthy subjects, elevations in fasting insulin levels (with normal fasting glucose levels) correspond to increased insulin resistance. Indeed, in nondiabetic subjects,1/(fasting insulin) is a well-known proxy for insulin sensitivity that decreases as subjects become more insulin resistant (and fasting insulin levels rise) .(Laako *et al.* 1993). However, insulin concentrations are not normally distributed. Thus, linear correlations between 1/(fasting insulin) and estimates of insulin sensitivity from the glucose clamp are not that strong. In addition, this index does not take into account the inappropriately low insulin secretion in the face of hyperglycemia seen in diabetic subjects or glucose-intolerant subjects. Consequently, using 1/ (fasting insulin) as a measurement of insulin sensitivity/resistance in patients with glucose intolerance or type 2 diabetes who have diminished pancreatic reserve leads to erroneous results.

GLUCOSE TO INSULIN RATIO -GLU0/INS0 (Glucose/insulin ratio): A number of studies (Silfen ME, *et al.* 2001,Vuguin P, *et al.* 2001) have used the fasting glucose/insulin ratio (G/I ratio) as an index of insulin resistance In the case of nondiabetic subjects, the G/I ratio is essentially functionally equivalent to 1/(fasting insulin) since fasting glucose levels are all in the normal range. However, the G/I ratio does not appropriately reflect the physiology underlying the determinants of insulin sensitivity (Quon MJ , *et al.* 2001). For example, given the same level of relative fasting hyperinsulinemia in

a diabetic and a nondiabetic insulin-resistant subject, 1/(fasting insulin) remains unchanged. However, under these same conditions, the G/I ratio paradoxically and erroneously increases in the diabetic subject. Therefore, the fasting G/I ratio is a conceptually flawed index of insulin sensitivity.

HOMA-(Homeostasis model assessment): Homeostasis model assessment (HOMA), developed in 1985 (Mathews DR, *et al.* 2001) is a model of interactions between glucose and insulin dynamics that is then used to predict fasting steady-state glucose and insulin concentrations for a wide range of possible combinations of insulin resistance and ß-cell function. Both the original HOMA and then updated HOMA2 assume a feedback loop between the liver and ß-cell (Wallace TM *et al.* 2004) i.e., glucose concentrations are regulated by insulin-dependent HGP, whereas insulin levels depend on the pancreatic ß-cell response to glucose concentrations. Thus, deficient ß-cell function reflects a diminished response of ß-cell to glucose stimulated insulin secretion . Likewise, insulin resistance is reflected by diminished suppressive effect of insulin on HGP. HOMA describes this glucose insulin homeostasis by a set of empirically derived nonlinear equations.

HOMA IR- IG/22.5

HOMA-IR - {[fasting insulin (μU/ml)]* [fasting glucose (mmol/l)]}/22.5. The denominator of 22.5 is a normalizing factor; i.e, the product of normal fasting plasma insulin of 5 U/ml and normal fasting plasma glucose of 4.5mmol/l typical of a “normal”

healthy individual = 22.5. Therefore, for an individual with “normal” insulin sensitivity,HOMA-IR = 1. HOMA-IR has a reasonable linear correlation with glucose clamp and minimal model estimates of insulin sensitivity/resistance in several studies of distinct populations (Radziuk J , *et al.* 2000). The coefficient of variation for HOMA-IR varies considerably depending upon the number of fasting samples obtained and the type of insulin assay used (Bonora E , *et al.* 2000).
Quantitative insulin sensitivity check index. Quantitative insulin sensitivity check index (QUICKI) is an empirically derived mathematical transformation of fasting blood glucose and plasma insulin concentrations that provides a reliable, reproducible, and accurate index of insulin sensitivity with excellent positive predictive power (Chen H *et al.* 2005).

Quantitative insulin sensitivity check index (QUICKI*)*. Like HOMA, QUICKI can be applied to normoglycemic and hyperglycemic patients. It is derived by calculating the inverse of the sum of logarithmically expressed values of fasting glucose and insulin:

$$\frac{1}{[\log(I0) + \log(G0)]}$$

Many investigators believe that QUICKI is superior to HOMA as a way of determining insulin sensitivity, although the two values correlate well. As the SI decreases, QUICKI values increase.

OGTT or a meal to determine insulin sensitivity/resistance (Cobelli C *et al.* 2007). Glucose disposal after an oral glucose load

or a meal is mediated by a complex dynamic process that includes absorption, glucose effectiveness, neuro hormonal actions, incretin actions, insulin secretion, and metabolic actions of insulin that primarily determine the balance between peripheral glucose utilization and HGP. Surrogate indexes that depend on dynamic testing take into account both fasting steady-state and dynamic postglucose load plasma glucose and insulin levels.

MCAULEY INDEX: The authors(MCAULEY *et al.* , 2001) proposed a formula for predicting insulin resistance in normoglycemic individuals. regression analysis was used to estimate the cut –off points and the importance of various data for insulin resistance(fasting concentration of insulin ,triglycerides, aspartate amino transferase ,BMI, waist circumference).A bootstrap procedure was used to find an index , corrected for fat –free mass obtained by hyperinsulinemic euglycemic clamp(Mffm/I).An insulin sensitivity obtained from HEC of $\leq$ 6.3 (expressed as glucose disposal rate in milligrams per kilogram per minute divided by average plasma insulin concentration in (mIU/l) and triglycerides(TAG,mmol/l) showed the best prediction of insulin resistance as follows:

$$\text{MCAULEY} - \text{Mffm/I} = e^{(2.63 - 0.2 * \ln(I0) - 0.31 \ln(TAG0))}$$

BELFOIRE INDEX: The condition for calculation of the belfoire formula is the definition of the normal value for basal glucose and insulin concentrations for mean normal value for glucose and insulin areas during OGTT(Belfoire *et al.* , 1998).the main point of the

belfoire formula is the comparison of insulin and glucose values measured .

BELFOIRE(OG) - 2/IAUC*GAUC+1

IAUC- Insulin area under the curve

GAUC-Glucose area under the curve.

(or) it can be written as $ISI_{Belfoire} – 2/Gs/G_N * I_S/I_N + 1$

Gs, G_N - plasma glucose concentrations expressed as fasting values or as obtained during a standard OGTT at 0 and 2h areas are equal to $G_{S,N} = G_0 + G_{120}$) or at 0,1 and 2 h (0-1-2h areas are equal to $G_{S,N}$ $= ½ (G_0 + G_{60} + G_{120})$;

IS,IN – Plasma insulin concentrations expressed as fasting values or areas obtained during a standard OGTT at 0 and 2h (0-1-2h areas are equal to $I_{S,N} = I_0 + I_{60} + I_{120}$).

The subscript S and N refer to “subjects” and “normal reference values”, respectively. insulin sensitivity calculated using these formulas can achieve only values between 0 and 2.In subjects with normal insulin sensitivity is it around 1; in overweight subjects ,in subjects with impaired glucose tolerance and with diabetes type 2 this value is below 1.correlation coefficient between between $ISI_{Belfoire}$ and HEC were in the original sudy reported to be 0.93-0.99 other authors found lower correlation coefficients of these formulas with HEC :0.65 ; in subjects with normal glucose tolerance,0.54; in subjects with impaired glucose tolerance, and 0.48 ; in subjects with diabetes type 2.

Matsuda: The index of whole body insulin sensitivity –proposed by Matsuda and Defronzo *et al.* (1999) combines both hepatic and peripheral insulin sensitivity. this index is calculated from plasma glucose (mg/dl) and insulin(mIU/ml). concentrations in fasting state and during OGTT. the correlation coefficients between ISI matsuda and HEC were 0.73 in subjects with normal glucose tolerance ,0.66 in subjects with impaired glucose tolerance and 0.60 in nondiabetic subjects. however in subjects with diabetes mellitus type 2 the correlation proved to be weaker 0.54.

MATSUDA - $10000/\sqrt{G*I*GAUC*IAUC}$

I0	-	fasting pasma insulin concentration
G0	-	fasting glucose concentration
Gmean	-	mean plasma glucose concentration during OGTT
I mean	-	mean plasma insulin concentration during OGTT
10000	-	Simplifying constant to get numbers from 0 to 12
√	-	correction of the nonlinear values distribution.

GUTTINDEX: [75.000+(fasglu-2hglu)*0.19*bw]/120min

The index correlates well with direct estimates of insulin sensitivity obtained from the glucose clamp study (GUTT M *et al.* 2000).In a large prospective study, this index was the best at

predicting onset of type 2 diabetes when compared with other surrogate indexes derived from dynamic tests (including Avignon, Belfiore, and Stumvoll)

Where the term- (0.19 *BW) denotes glucose space, and BW is body weight (kg).

Mean of the 0- and 120-min glucose values from the OGTT). The mean serum insulin (MSI, mU/l) is the mean plasma insulin concentrations obtained from the 0- and 120-min samples of the OGTT.

CEDERHOLM INDEX: The insulin sensitivity index proposed by cederholm and wibell *et al.*, 1990) represents peripheral insulin sensitivity and muscular uptake, due to the dominant role of peripheral tissues in glucose disposal after an oral glucose load.

$$\textbf{CEDERHOLM} - \frac{75000+(G2HG)*(1.15*180)*0.19*m)}{120* Gmean *\log (I mean)}$$

where

75000 - oral glucose load in an OGTT in mg.

G_0 - fasting plasma glucose concentration (mmol/l),

G_{120} - plasma glucose concentration in the 120^{th} min of OGTT(mmol/l),

1.15 - factor transforming whole venous blood glucose

to plasma values (not necessary ,if glucose concentration is estimated in plasma),

180 - Conversion factor to transform plasma glucose

concentration from mmol/l in to mg/l,

0.19 - glucose space in liter per kg of weight,

m - Body weight(kg),

120 - Duration of OGTT(min)

I_{mean} - Mean plasma insulin concentration during OGTT(mU/l)

G_{mean} - mean plasma glucose concentration during OGTT(mmol/l).

values found in normal non-obese individuals were reported to be about 79 $\pm$ 14 $mg.l^2$ $.mmol^{-1}.mU^{-1}.min^{-1}$,lower in obese individuals , in subjects with impaired glucose tolerance and in patients with type2 diabetes. Determined correlation coefficients of this index with HEC: 0.52 in subjects with normal glucose tolerance, 0.48 in subjects with impaired glucose tolerance and 0.40 in subjects with diabetes type2.

Other authors (stumvoll *et al.*, 2000) determined correlation coefficients of this index with HEC and found to be 0.60 in non –diabetic subjects.

AVIGNON INDEX: The authors (Avignon *et al.* , 1999) proposed 3 insulin sensitivity indices: Sib (derived from fasting plasma insulin and glucose concentrations) , Si2h

(derived from plasma insulin and glucose concentrations in the 120th min of OGTT) and Sim (derived by averaging Sib and Si2h after balancing by a coefficient of 0.137 to give the same weight to both indices).

AVIGNON-[(0.317*SiB)+Si2h]/2

1. SiB 108/I0*G0*VD
2. Si2h 108/I120*G120*VD
3. VD 150*BW

I and G represent the plasma concentrations of insulin(mIU/l)and glucose (mmol/l) respectively. VD is the glucose distribution volume calculated using a monocompartmental model: VD=150ml/kg of body weight.

The SiM index correlated well with insulin sensitivity obtained during insulin – modified frequently sampled intravenous glucose tolerance test (FSIVGTT) (Bergman *et al.* , 1987) in individuals with normal glucose tolerance (0.89) , with impaired glucose tolerance (0.96), and in patients with diabetes mellitus type 2 (0.83). it is important to note that other indices were correlated with the clamp method , seen as the “gold standard” in estimating insulin sensitivity. The correlation coefficients of FSIVGTT and the euglycemic clamp were reported to be 0.84(Beard *et al.*, 1986), 0.89 and 0.62 (Saad *et al.* , 1994),diminishing the weight of Avignon indices to the level of other indices discussed above.

INSULIN SECRETION / RELEASE:

BETA CELL FUNCTION: It is well established that abnormalities in insulin secretion are an important determinant of diabetes mellitus and other states of glucose intolerance. However, assessment of β-cell function in humans under physiological conditions has been a challenge because of its complex interplay with other key system variables. Although β-cell function commonly is inferred from plasma insulin concentrations, this approach introduces error due to the confounding effect of hepatic insulin extraction. On the other hand, concordant measurement of C-peptide and insulin concentrations enables simultaneous assessment of insulin secretion and hepatic insulin extraction. An additional difficulty is that measurement of insulin secretion alone provides limited insight because appropriateness of β-cell function must be interpreted relative to the prevailing level of insulin action. Since the primary goal of most studies is to determine how alterations in β-cell function as well as in insulin action and hepatic insulin extraction influence carbohydrate, fat, and protein metabolism, ideally these should be assessed under physiological conditions by using a single, simple physiological test, i.e., in the presence of glucose, amino acids, incretins, and neural signals. Models of insulin secretion enable evaluation of beta cell function following intravenous injection of bolus of glucose during

IVGTT or after ingestion of glucose during OGTT or a mixed meal. The oral perturbation are more physiological than intravenous once with incretin effect in operation and with meal being superior to OGTT because of presence of nutrients. Insulin action and hepatic extraction also can be assessed by models during IVGTT and OGTT meal.

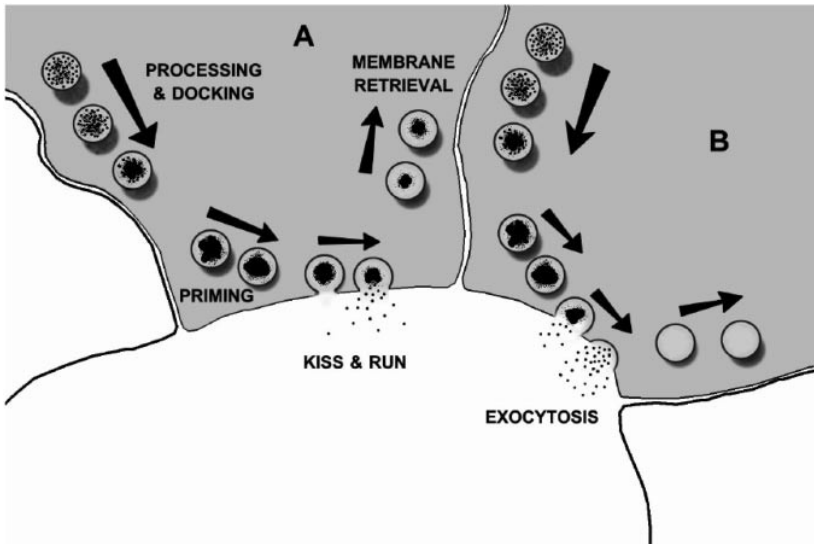


Fig:2 Representative β -cells discharging insulin into an islet capillary loop. Major steps preceding insulin secretion are synthesis (not shown), proinsulin processing to insulin and vesicle transport and docking to membrane, vesicle priming, and then insulin discharge from vesicle into capillary as either a fraction of insulin contents (kiss and run illustrated in cell A) or all insulin contents (exocytosis in cell B).

INSULIN SECRETION INDICES:

Table 3: Insulin Secretion Indices

Index	Equation
HOMA B	$20\ I_0/G_0-3.5$
STUMVOLL	0.22-0.0032*BMI-.0000645 *2hi-0.0037*G90
½ II	$I½-\ I_0/\ G_0½-\ G_0$
2h II	ΔIAUC/ΔGAUC

HOMA-S	3.33 I_0/ G_0-3.5

HOMA B - 20 I_0 /G_0-3.5 Computer simulations have been used to generate a normogram from which mathematical transformations of fasting glucose and insulin data from individual subjects determine unique combinations of SI (HOMA%S) and ß-cell function (HOMA%B) from steady-state conditions. The updated HOMA2 accommodates assessment of HOMA%S and HOMA%B in subjects with glucose levels ≤25 mM, accounts for renal glucose losses, assumes reduced suppression of HGP and increased insulin secretion in response to glucose levels >10 mM, and allows for the use of total or specific insulin assays. An important caveat for HOMA%B (determined from fasting glucose and insulin concentrations) is that it imputes a dynamic ß -cell function (i.e., glucose stimulated insulin secretion) from fasting steady-state data.

Where I_0 - fasting insulin (μU/ml)

G_0 - fasting glucose (mmol)

HOMA2 model generates model-derived estimates of %B and %S, rather than linear approximations. It took account of

1. Variations in hepatic & peripheral glucose resistance,
2. Increases in the insulin secretion curve for plasma glucose concentrations above 10 mmol/L (180 mg/dL) and
3. The contribution of circulating proinsulin .

The model was recalibrated also to give %B and %S values of 100% in normal young adults when using currently available assays for insulin, specific insulin or C-peptide.

STUMVOLL INDEX: (Stumvoll *et al.*, 2001) proposed a series of indices (approximately 10) calculated from plasma glucose (mmol/l) and insulin (pmol/l) concentrations during OGTT. The equations were generated using the multiple linear regression analysis and adapted to the availabilities of sampling time during OGTT and of demographic parameters (BMI).their correlation coefficients with HEC were in range between 0.62 and 0.79.

STUMVOLL - 0.22-0.0032*BMI-0.0000645*2hI-0.0037*G90

β-CELL FUNCTION FROM BASAL MEASUREMENTS

The easiest and thus most popular assessment of β-cell function is the homeostatic responsively index HOMA-B, derived from basal measurements of insulin and glucose (Matthews DR *et al.* 1985) HOMA-B is widely used because of its simplicity, it is worth pointing out that it reflects the release of insulin under nonstimulated conditions. The homeostatic model HOMA-S also provides an index of insulin sensitivity under nonstimulated conditions, thus permitting evaluation of ß-cell function in relation to the prevailing insulin action. It should be noted that an hyperbolic relationship between the two indexes is inherent in the calculation, since HOMA-B * HOMA-S is a function of basal glucose concentration such that the two measurements are, by definition, in a

given subject hyperbolically inversely related provided basal glucose [and also basal insulin if the computer model HOMA-S (Levy JC *et al.* 2004) is used] remains constant (Caumo A ,*et al.* 2006).

Beta cell function and insulin sensitivity can be assessed by both IVGTT and OGTT minimal model indexes:

Hepatic Insulin Extraction: (FROM IVGTT):

Since plasma insulin concentrations are measured during the IVGTT for estimating insulin sensitivity, posthepatic insulin delivery rates can be calculated allowing estimation of hepatic insulin extraction during the test, HE_{ivgtt}, and in basal condition, Heb(Toffolo G *et al.* , 2006) . To this end, the protocol of choice is an insulin-modified IVGTT, since the decay of insulin concentration observed after exogenous insulin administration facilitates a reliable estimation of insulin kinetics. However, a population approach, similar to that developed for C-peptide kinetics, is being developed for insulin kinetics, and preliminary results are very encouraging (Campioni M *et al.*, 2004).

First- and Second-Phase Indexes and Delay Based on C-Peptide:

AIR has two major limitations. First, it is a composite "β -cell function and hepatic insulin extraction" index, and second, it probes ß -cell behavior in a very brief time window and immediately after a markedly supraphysiological glucose stimulus. ß-Cell function can be assessed from plasma glucose and C-peptide concentrations measured during a standard or insulin- modified IVGTT by using the

minimal model. Insulin secretion is made up of two components, first- and second-phase secretion .First phase introduces a derivative control, since it is proportional to the rate of increase of glucose from basal up to the maximum through the parameter $Ø_1$, which defines the first responsively index (in contrast to AIR,$Ø_1$ is a pure ß-cell function index; correlation between AIR and $Ø_1$ in 204 individuals is 0.72). Second-phase insulin secretion is believed to be derived from the provision and/or docking of new insulin secretory granules that occurs in response to (i.e. proportional to) a given glucose concentration through the parameter $Ø_2$, which defines the second-phase responsively index, and reaches the releasable pool with a delaytime constant, T.

IVGTT Minimal Model Indexes

BETA CELL RESPONSIVITY:

- Basal $Ø_b(min^{-1})$ – basal secretion per unit basal glucose concentration
- 1st phase $Ø_1$ $(10^{-9}min^{-1})$ – amount of first phase secreted insulin per unit increase of glucose concentration.
- 2nd phase $Ø_2$ $(10^{-9}min^{-1})$ - over basal average second phase insulin secretion per unit over basal average glucose concentration.
- Delay T (min) – delay between 2nd phase secretion and glucose concentration.
- Total $Ø_{ivgtt}(10^{-9}min^{-1})$ – over all responsivity from $Ø_1$ and $Ø_2$

- SI $_{ivgtt}$ (10^{-5} min^{-1} per pM) – effect of insulin to stimulate glucose disposal and inhibit glucose production.

DISPOSITION INDEX

- 1st phase DI_1 (10^{-14} dl.kg $^{-1}$ min^{-1}) – $Ø_1 * SI_{ivgtt}$
- 2nd phase DI_2 (10^{-14} dl.kg $^{-1}$ min^{-1}) – $Ø_2 * SI_{ivgtt}$
- Total DI_{ivgtt} (10^{-14} dl.kg $^{-1}$ min^{-1}per pM) – $Ø_{ivgtt} * SI_{ivgtt}$

HEPATIC INSULIN EXTRACTION

- He_b (%) – basal insulin extraction minus basal post hepatic delivery rate over basal insulin secretion.
- HE $_{ivgtt}$(%) – average insulin secretion minus average post hepatic delivery over average insulin secretion during ivgtt.

There is also ogtt minimal model indices for calculating beta cell responsivity and insulin sensitivity sama as that of IVGTT minimal model indices.

Insulin secretion is the main defect seen in prediabtes stages and in maturity onset of diabetes in young.

PREDIABETES: The prediabetes stages are of three types:

1. IFG – IMPAIRED FASTING GLUCOSE
2. IGT- IMPAIRED GLUCOSE TOLERANCE
3. combined IFG and IGT

The term prediabetes refers to subjects with impaired glucose and /or impaired fasting glucose tolerance or combined IFG/IGT who are at increased risk for type 2

diabetes mellitus. Although both types of patients are at increased risk for developing type 2 diabetes mellitus and cardiovascular disease, they manifest distinct metabolic abnormalities. (Muhammad A. Abdul –ghani , *et al.* 2009). During past decade ,physiological studies have established that IFG and IGT are caused by different abnormalities in insulin secretion and action.

EPIDEMIOLOGY OF PREDIABETES:

- Epidemiologic studies demonstrated that IFG (110-125mg/dl) has lower prevalence than IGT because most of the studies were done prior to 2003, an fpg concentration of 110mg/dl(6.1Mm) was used as lower cut off point defining IFG.
- A minority of subjects with IGT (20%-25%) had concentration an fpg concentration of more than 110mg/dl(6.1Mm)
- Over half subject with IFG had 2hr pg concentration of less than 140mg/dl (7.8Mm) (Muhammad A. Abdul –ghani et al 2009).
- The absolute prevalence of IFG and IGT are ethnic dependent.
- For example the prevalence of IGT varies from as low as 6.3% in Chinese population (Chan JC *et al.,* 1997) to as high as 20.3% in a Swedish population (Larson H *et al.,* 1998)
- IGT is more frequent in women whereas the prevalence IFG is more than two fold higher in men (Nakagami *et al.,* 2003).

- IFG and IGT differ not only in their prevalence in the general population but also in their age and sex distribution.

Table 4: Pathophysiology of Prediabetes Stages

PATHOPHYSIOLOGY	IFG	IGT	IFG/IGT
Muscle a. Insulin sensitivity	Unaltered	Reduced	Reduced
Liver a. Insulin sensitivity	Reduced	Unaltered	Reduced
b. Hepatic glucose production	Elevated	Unaltered	Elevated
Pancreas a. First phase insulin response	Reduced	Reduced or unaltered	Reduced
b. Disposition index*	Reduced	Reduced	Reduced
c. Glucagon secretion	Elevated	Elevated	Elevated
Gut a.Glp-1 secretion	Reduced or elevated	Reduced or unaltered	Not studied
b.Gip secretion	Unaltered	Reduced or unaltered	Not studied
Adipose tissue a. Insulin sensitivity b. NEFA release	Reduced Unaltered	Reduced Elevated	Not studied Not studied
Adipocytokine release a. Brain b. kidney	Not studied Not studied	Not studied Not studied	Not studied Not studied

Table 5: Effects of aetiological factors on FPG levels (the development of IFG) ,2hPGlevels(the development of IGT),and combined FPG/2hPG levels (the development of IFG/IGT)

Ateiology	FPG levels -IFG	2h PG levels-IGT	FPG/2hPG-IFG/IGT
Environmental factors			
a. Physical activity	No effect	Increase	Not studied
b. Low dietary quality	No effect	Increase	No effect
c. Smoking	Increase	No effect or increase	Increase
Heritability			
a. Family history of diabetes	Increase	No effect	Increase
b. TCF7L2	Increase	Increase	Not studied
C. MTNR1B	Increase	No effect	Not studied
D. GCK	Increase	Increase	Not studied
E. GCKR	Increase	No effect	Not studied
F. G6PC2	Increase	No effect	Not studied
G. FTO	Increase	No effect	Not studied
H. PPARG	Increase	No effect	Not studied
Sex and anthropometry			
a. Male sex	Increase		No effect
b. Low birth weight	Increase		Not studied
c. Short adult stature	No effect		Not studied

Maturity onset diabetes of the young

Maturity onset diabetes of the young (MODY (NIH) refers to any of several hereditary forms of diabetes caused by mutations in an autosomal dominant gene (sex independent, i.e. inherited from any of the parents) disrupting insulin production. Unlike the polygenic recessive types 1 and 2 of diabetes caused by mutations in genes inherited from both parents, MODY is monogenic and easier

to manage than polygenic ones. As of 2004, six types have been enumerated, but more are likely to be added. MODY 2 and MODY 3 are the most common forms. The severity of the different types varies considerably, but most commonly MODY acts like a very mild version of type 1 diabetes, with continued partial insulin production and normal insulin sensitivity. MODY may be confused with type 1 or type 2 diabetes. It is not young-onset type 2 diabetes (in a young person), as might erroneously be inferred from the name.

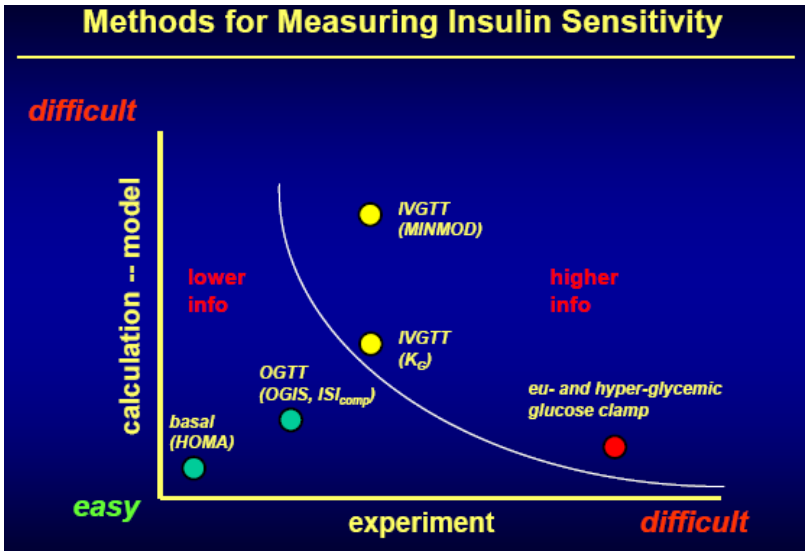
Table 6: Classification of different types of MODY (Maturity onset of diabetes of the young)

Mody types	Chromo - somes	Gene	Omim (gene)	Omim (pheno)	Frequency	Clinical Features	Therapeutic Intervention
Mody1	20	HNF4A	600281	125850	Rare	Macrosomia Transient hyperinsulinemic hypoglycemia, Familial hyperlipidemia	Insulin, sulphonyl urea
Mody2	7	GCK	138079	125851	Frequent	Mild insulin deficiency, low Birth weight infants, neonatal diabetes mellitus in homozygous	Diet, exercise
Mody3	12	HNF1A	142410	600496	Frequent	Pancreatic exocrine failure, Increased sensitivity to sulphonyl urea	Insulin
Mody4	13	IPF1	600733	606392	Rare	Pancreatic agenesis	Insulin
Mody5	----	HNF1ß	189907	137920	Frequent	Agenesis of pancreatic tail and body, pancreatic exocrine failure	Insulin
Mody6	-----	NEUROD1	601724	606394	Rare	Pancreatic anomalies	Insulin

Mody7	----	KLF11	610508	----	Rare	Pancreatic malignancy	Insulin
Mody8	----	CEL	609812	----	Rare	Pancreatic exocrine and endocrine failure	insulin
Mody9	----	PAX4	612225	----	Rare	Diabetes mellitus	-----
Permanent Neonatal Diabetes mellitus	-----	KCNJ11	606176	-----	------	-------	------
Transient Neonatal diabetes mellitus	-----	ABCC8	601410 610374 610582	----	-----	Some forms of neonatal diabetes are permanent	----

METHODS FOR MEASURING INSULIN SENSITIVITY:

Insulin sensitivity is used to assess hepatic insulin sensitivity and peripheral insulin sensitivity. The concept of insulin resistance is relatively easy to understand, but determining precisely who is insulin resistant is more complicated. The relationship between glucose and insulin is quite complex and involves the interaction of many metabolic and regulatory factors.



Direct measures of insulin sensitivity

1. Hyperinsulinemic Euglycemic Clamp Technique

2. Insulin Suppression Test

Hyperinsulinemic euglucemic clamp technique (GOLD STANDARD TECHNIQUE): this glucose clamp technique originally developed by (DeFronzo *et al.* 1936) ,is a widely accepted as the reference standard for directly determining metabolic insulin sensitivity in human.

Procedure and concept:

After an overnight fast ,insulin is infused intravenously at a constant rate that may range from 5 to 120 mU.m-2.min-1(dose per body surface area per minute).this constant insulin infusion results in a new steady state insulin level that is above fasting level (hyperinsulinemic).As a consequence ,glucose disposal in skeletal muscle and adipose tissue is increased ,whereas HGP is suppressed .under these conditions ,a bed side glucose analyzer is used to frenquently monitor blood glucose level at 5 to 10-min intervals while 20% dextrose is given intravenously at a variable rate to “clamp” blood glucose concentration in normal range(euglucemic). an infusion of potassium phosphate is also given to prevent hypokalemia resulting from hyperinsulinemia and increased glucose disposal.the whole body glucose disposal at a given at a given level of hyperinsulinemia can be determined directly alternatively an insulin sensitivity index (S1) derived

from clamp data can be defined as SI clamp=M/(G*ΔI),where M is normalized for G(steady state blood glucose concentration) and ΔI (difference between fasting and steady state plasma insulin concentration) (Katz A *et al.* 2000).

Advantages:

The main advantage of using glucose clamp to estimate insulin sensitivity /resistance in humans is that it directly measures whole body glucose disposal at a given level of insulinemia under steady state conditions.

Limitations:

- Time consuming
- Labor intensive
- Expensive
- It requires an experienced operator to manage technical difficulties. Another limitation is that the clamp utilizes steady state insulin level that may be supraphysiological.

Insulin Suppression Test: This is a another method that directly measures metabolic insulin sensitivity/ resistance, was introduced by Shen *et al.* (Shen SW, *et al.* 1970) and subsequently modified by Harano *et al.* (Harano Y, *et al.* 1978). After an over night fast, somatostatin (250 µg/h) is intravenously infused to suppress endogenous secretion of insulin and glucagon. Simultaneously insulin and glucose are infused into same anticubital vein for 3h. From the contra

lateral arm, blood samples for glucose and insulin determination are taken at every 30mins for 2.5h and then at 10mins interval from 150-180 mins IST. The constant infusions of insulin and glucose will determined steady state plasma insulin (SSPI) and glucose (SSPG) concentrations. The steady state period is assumed to be from 150-180 mins after initiation of IST. SSPI concentrations are generally similar among subjects. Therefore, the SSPG concentration will be higher in insulin resistance subjects and lower in insulin sensitive subjects; ie SSPG values are inversely related to insulin sensitivity. The IST provides a direct measure (SSPG) of the ability of exogenous insulin to mediate disposal of an intravenous glucose load under steady state conditions where endogenous insulin secretion is suppressed.

Advantage:

- The SSPG is highly reproducible direct measurement of metabolic actions of insulin that is less labor intensive and less technically demanding than the glucose clamp.
- Estimates of insulin sensitivity determined by SSPG correlate well with reference standard glucose clamp estimates in normal subjects and in patients with type 2 diabetes mellitus.
- More over, the IST can be used for larger populations that may pose difficulties for application of the glucose clamp (Yeni-Komshian H *et al.* 2000) .

Limitation

Many of the limitations of the IST are similar to those described above for the glucose clamp (with the exceptions that the IST is less technically demanding). Thus, it is impractical to apply the IST in large epidemiological studies or in the clinical care settings.

Indirect Measures of Insulin Sensitivity

Minimal model analysis of frequently sampled intravenous glucose tolerance test

Procedure and concept

The minimal model, developed by (Bergman, R N *et al.* 1979), provides an indirect measurement of metabolic insulin sensitivity/ resistance on the basis of glucose and insulin data obtained during a frequently sampled intravenous glucose tolerance test. After an over night fast, an intravenous bolus glucose is infused over 2 mins starting at time 0 currently, a modified FSIVGTT is used where exogenous insulin is also infused over 5 mins beginning 20 mins after the intravenous glucose bolus (Quon M J *et al.* 2001). Blood samples are taken for plasma glucose and insulin measurement at 0-180 mins (10 mins interval). These data are then subjected to minimal model analysis using the computer program MINMOD to generate and index of insulin sensitivity(SI).

Advantage:

- Minimal model analysis of the modified FSIVGTT is easier than the glucose clamp method because it is slightly less labor

intensive, steady state conditions are not required, and there are no intravenous infusion that require constant adjustment.

- The minimal model generates excellent prediction of glucose disappearance during the FSIVGTT.

Limitations:

- It still involves intravenous infusions with multiple blood sampling over a 3h period that are nearly as labor intensive as the glucose clamp or IST.
- The over simplification of the minimal model involves lumping together effects of insulin to promote peripheral glucose utilization suppress HGP.

Insulin tolerance test (ITT). A simplified version of IST, ITT measures the decline in serum glucose after an IV bolus of regular insulin (0.1–0.5 U/kg) is administered. Several insulin and glucose levels are sampled over the following 15 minutes (depending on the protocol used). The ITT primarily measures insulin-stimulated uptake of glucose into skeletal muscle. Because this test is so brief, there's very little danger of counter-regulatory hormones interfering with its results. IV access should be established for insulin injection, blood sampling, and for rapid administration of D50W should severe hypoglycemia occur. Normal values for women with PCOS have not been published to date, but normal ranges for insulin sensitivity in a general population have been published for persons with a body mass index below 30 kg/m2 and for obese subjects (BMI >30 kg/m2) at 0.026 to 0.085 mmol/L /minute and 0.012 to 0.017

mmol/L /minute respectively. These values reflect the rate of decline of log transformed glucose values.

Continuous infusion of glucose with model assessment (CIGMA). Like ITT, CIGMA requires fewer venipunctures and is less laborious than clamp techniques. A constant IV glucose infusion is administered, and samples for glucose and insulin are drawn at 50, 55, and 60 minutes. A mathematical model is then used to calculate SI. The results are reasonably compatible with clamp techniques; however, few laboratories have used CIGMA for insulin sensitivity testing in diabetic patients and there is no substantive data using the CIGMA technique in women with PCOS.

Oral Glucose Tolerance Test/ Meal Tolerance Test

The oral glucose tolerance test (OGTT) is a simple test widely used in clinical practice to diagnose glucose intolerance and type 2 diabetes (American diabetes association 2007). After over night fast, blood samples for determinations of glucose and insulin concentrations are taken at 0, 30,60 and 120 mins following a standard oral glucose load (75 g) or a standard meal (Dalla Man C, *et al.* 2005). Oral glucose tolerance reflects the efficiency of the body to dispose of glucose after an oral glucose load or meal. The OGTT or meal tolerance test mimics the glucose and dynamics of physiological conditions more closely than conditions of the glucose clamp, IST or FSIVGTT. In addition to metabolic actions of insulin, insulin secretion, incretin effects, and other factors contribute importantly to glucose tolerance.

Table 7: Protocols: their attributes, and information content

Protocol	Is It Physiological?	Is It Simple?	Can It Assess β-Cell Function?	Can It Assess Insulin Sensitivity?	Can It Assess Hepatic Insulin Extraction?
Basal State	Yes	Yes	Yes, but limited	Yes, but limited	No
Intravenous perturbation i) Hyperglycemic clamp	No	No	Yes, but limited without a model	Yes, but requires a model	Yes, but requires a model.
ii) Euglycemic clamp	No	No	No	Yes	No
iii) IVGTT	No	No	Yes, but limited without a model	Yes, but limited without a model	Yes, but requires a model.
iv) Graded infusion	No	No	Yes, but limited without a model	Yes, but requires a model.	Yes, but requires a model.
Oral perturbation i) OGTT	Yes, but no nutrients	Yes	Yes, , but limited without a model	Yes, but requires a model.	Yes, but requires a model.
ii) Meal	Yes	Yes	Yes, but limited without a model	Yes, but requires a model.	Yes, but requires a model.

ORAL GLUCOSE TOLERANCE TEST: The oral glucose tolerance test (OGTT) has traditionally been used to classify the status of glucose tolerance for diagnostic purposes: normal glucose tolerance (NGT) versus impaired glucose tolerance (IGT) versus diabetes (WHO). More recently, however, some authors have attempted to exploit the information contained in a 2-h OGTT to estimate insulin sensitivity (Mari A, *et al.* 2001) and ß-cell function .

While the derived indexes are less accurate than the respective gold-standard methods, they can be obtained more easily and used in large epidemiological or genetic association studies. These indexes take advantage of glucose and insulin concentrations at specific time points during the OGTT. the glucose curve during the OGTT as "biphasic," "domed," and "upward" (Fuchigami M, *et al.* 1994). The main finding was that in patients with type 2 diabetes, the prevalence of biphasic was lower and the prevalence of upward was higher than in any other group. This appears somewhat trivial, because the category upward naturally favors enrichment with diabetic subjects who, by definition, have the highest glucose concentrations at the end of the OGTT. Nevertheless, it is interesting to note that the biphasic shape was most strongly associated with NGT in that study. This suggests that the shape harbors metabolic information not captured by the level of glycemia alone.

Definition of monophasic and biphasic plasma glucose curve shapes:

- ✓ The glucose curve shape of an OGTT was classified as "monophasic" when plasma glucose increased after an oral glucose load to the maximum after 30–90 min and decreased until 120 min with a final downward move of at least 0.25 mmol/l between 90 and 120 min.
- ✓ Glucose shapes that reached a nadir after an initial increase and increased again >0.25 mmol/l until 120 min were classified as "biphasic"

- ✓ The shape index, calculated as glucose at 90 min (Gluc90) minus glucose at 120 min (Gluc120) was treated as a continuous variable in correlational analyses. A shape index >0 indicates biphasic and a shape index < 0 indicates monophasic.
- ✓ In subjects showing a decrease of plasma glucose between 30 and 60 min, an increase between 60 and 90 min, and a decrease again between 90 and 120 min (i.e., two complete peaks or a “triphasic” shape), the increase between 60 and 90 min was taken as shape index to avoid false classification of these subjects as monophasic. One IGT subject with a continuous increase during the 120 min was excluded.

LITERATURE SURVEY

R S Scott *et al*., (2009) made a study on "**comparison of indices of insulin resistance with metabolic syndrome classifications to predict the development of impaired fasting glucose in over weight and obese subjects: a 3- year prospective study".** The study was done to compare the ability of biochemical indices of insulin resistance (ir) with metabolic syndrome (MetS) classifications to predict changes in blood glucose control over a 3-year period in over weight and obese subjects. The study concluded that IR indices derived from plasma triglyceride concentration, were sensitive predictors for the development of IFG risk group in normoglycemic overweight and obese subjects and indices derived from glucose and insulin did not identify at this group. this study also showed that presence of MetS and its abnormalities of an increased trig:HDL ratio and low plasma adiponectin concentration were all sensitive predictors of IFG.

Maffeis Claudio *et al*.**,** (2009) performed a study on "**Fasting plasma glucose (FPG) and the risk of impaired glucose tolerance in obese children and adolescents."** The study was done to assess whether one or more biochemical indexes measured in fasting conditions could be used to identify obese children at risk of IGT.this study was carried out in 563 obese children and adolescents (M/F -315/248; aged 4-17 years)was recruited and underwent anthropometric evaluation and OGTT.Anthropometric parameters,

fasting plasma glucose(FPG),fasting serum insulin(FSI),and homeostatic model assessment of insulin resistance (HOMA-IR) were tested in pursuit of a possible threshold to be used as a predictor of IGT.Results shown was that the three parameter did not show significantly different sensitivity/specificity in the pooled population or in the gender /puberty subgroups. In this study they concluded that a gender/puberty independent cut off of FPG may be considered as screening tool to narrow clinical indication to OGTT in obese white children and adolescents.

Malita FM *et al*., (2009) done a study on "**comparison between several insulin sensitivity indices and metabolic risk factors in overweight and obese postmenopausal women-A MONET study."** The purpose of this study was to compare the relationship of several insulin sensitivity indices with cardiometabolic risk factors in overweight and obese postmenopausal women. They concluded that the present study indicates that the different methods of measuring and/or expressing insulin sensitivity display variations for associations with cardiometabolic risk factors. Therefore interpretations of relation ships between insulin sensitivity indices and cardiometabolic risk factors should take into account the method used to estimate and express insulin sensitivity.

Jens J. Holst *et al.,* (2009) made a study on **"Natural history of insulin sensitivity and insulin secretion in the progression from normal glucose tolerance to impaired fasting glycemia and**

impaired glucose tolerance: the inter99 study" the aim of the study was to describe the natural history of insulin secretion and insulin sensitivity in the development of impaired fasting glycemia(IFG),impaired glucose tolerance(IGT),and combined IFG/IGT. in this study baseline and 5-year follow up data from the Inter 99 study were used.individuals with normal glucose tolerance (NGT) at baseline and IFG and IGT ,combined IFG/IGT, or NGT at the 5-year follow up were examined with an ogtt . Insulin sensitivity index(ISI),HOMA-IS, early phase insulin release (EPIR),and insulin secretion relative to insulin action (disposition index) were estimated. The conclusion of this study is that there is a stationary reduced insulin secretion followed by decline in hepatic insulin sensitivity characterizes transition from NGT-IFG.in case of IGT there is low whole body insulin sensitivity with a secondary lack of ß-cell function. Thereby both IFG and IGT have different mechanisms which will be useful for the prevention and treatment of the diabetes that succeeds them.

K.Faerch *et al.,* (2009) attempted a study on "P**athophysiology and aetiology of impaired fasting glycemia and impaired glucose tolerance: does it matter for prevention and treatment of type2 diabetes."**the aim of the study was to understand the aetiology and pathophysiology of the prediabetic states might give a basis for the development of individualized prevention and treatment strategies for type 2 diabetes. Prediabetic stages are IFG, IGT, combined IFG/IGT. These stages have different

pathophysiology and aetiology. So in this study they concluded that the transition from the prediabetic states to overt type 2 diabetes is characterized by a non –reversible vicious cycle that include severe delitorius effects on glucose metabolism, there are good reasons to use the well established aetiological and pathophysiological differences in IFG, IGT and IFG/IGT to design individualized preventive strategies.

A.Vaag *et al.,* (2008) done a study on "**Impaired fasting glycemia vs impaired glucose tolerance:similar impairment of pancreatic alpha and beta cell function but differential roles of incretin hormones and insulin action."** The hypothesis of the study was to prevent type 2 diabetes in impaired fasting glycemia (IFG) vs impaired glucose tolerance (IGT) may differ depending on the underlying pathophysiology. They evaluated insulin secretion during OGTTs and IVGTTs, hepatic and pheripheral insulin action and glucagon and incretin hormone secretion in individuals with IFG and IGT and NGT (normal glucose tolerance)finally in the study they concluded that differentiated preventive initiatives in prediabetic individuals should be tested, targeting the specific above mentioned metabolic defects.

M.Lassko *et al.,* (2008) done a study on "**Insulin sensitivity, insulin release and glucagon-like peptide-1 levels in persons with impaired fasting glucose and/or impaired glucose tolerance in the EUGENE2 study."** the aim of the study was to examine the phenotype of the individual impaired fasting glucose (IFG) and/or

impaired glucose tolerance(IGT) with regard to insulin release and insulin resistance. In their study they took non diabetic offspring (n=874;mean age 40±10.4 years; BMI 26.6±4.9 kg/m^2) of type 2 diabetic patients from different European centers were examined with insulin sensitivity(euglycemic clamp),insulin release (IVGTT) and glucose tolerance (OGTT)levels of glucagon like peptide-1(GLP-1) and gastric inhibitory polypeptide (GIP) were measured during ogtt. They conclude that primary mechanism leading to hyperglycemia in participants with IFG is likely to be impaired basal and first –phase insulin secretion, whereas in IGT the primary mechanism leading to post glucose hyperglycemia is insulin resistance reduced GLP-1 levels were seen in all groups with abnormal glucose tolerance and were unrelated to the insulin release pattern during an IVGTT.

Ranganath Muniyappa *et al*., (2007) made a study on **"Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations and appropriate usage."** the aim of this study was to highlight merits, limitations and appropriate use of current invivo measures of insulin sensitivity/insulin resistance. quantifying insulin resistance/ sensitivity in humans and animal models is of great importance for epidemiological studies. There are several direct and indirect methods for assessing insulin sensitivity/resistance. But these methods are labor intensive, time consuming. And it is important to understand the concepts underlying each method so that relative

merits and limitations are appropriately matched to proposed applications. And they concluded the study by regarding simple surrogates, QUICKI and log (HOMA) are among best and most extensively validated.

James D Johnson *et al.,* (2007) made a study on **"pancreatic apoptosis in maturity onset diabetes of the young."** maturity onset diabetes (MODY) denotes a group of disorders characterized by an autosomal dominant mode of inheritance. Mody presents in children, adolescents, or young adults and may account for up to 5% of diabetes cases. Gene mutated in mody include 5 transcription factor and metabolic enzyme glucokinase ; apart from their involvement in the function of pancreatic beta cells, these genes also have several unexpected common ties. The aim of this study was evidence from recent studies that suggests important roles for mody genes in the control of beta cell metabolic pathways and cell fate. From this study they concluded that investigation in to the pathophysiology of diabetes have been guided by the discovery of genes associated with the disease. The study have also suggested that mutation in HNF-1alpha, HNF-4alpha or PDX-1 may predispose certain populations to late onset type2 diabetes. The evidence is that mutation in mody genes play a dominant role in the most common forms of type 2 diabetes is lacking.

Mehtap Cakir *et al.,* (2006) made a study on **"Reproducibility of fasting and ogtt-derived insulin resistance indices in normoglycemic women"** the aim of this study was to

determine the reproducibility of fasting and oral glucose tolerance test (ogtt) – derived insulin resistance (IR) indices in obese and non obese women in this study they used twenty one obese (BMI 37.7±6.3 kg/m) and non obese (BMI 21.5±1.0kg/m)age-matched, healthy, premenopausal women were included in the study. An ogtt was performed twice, with a 1-week interval between tests.IR was also calculated from fasting and post load glucose and insulin values, using some of the more well-known indices. In this they concluded that when two groups were evaluated separately all indices were reproducible in obese subjects ,but some indices were not reproducible in non obese healthy controls. When results were analyzed in the study population as a whole, all indices were reproducible.

Supamai Soonthurnpun *et al.,* (2003) done a study on **"Novel insulin sensitivity derived from oral glucose tolerance test"** the euglycemic hyperglycemic clamp is generally regarded as a reference method for assessing insulin sensitivity. However this method is laborious and expensive. The oral glucose tolerance, the most commonly used method for evaluating whole body glucose tolerance and insulin sensitivity. in the previous study the correlation between ISI(ogtt) and ISIclamp may not be satisfactory.this is because ISIclamp is designed for measuring pheripher al glucose utilization, whereas plasma glucose responses during ogtt are results pheripheral glucose utilization and hepatic glucose production based on this problem they developed an equation of ISI(ogtt). They tested

with healthy volunteers and finally they concluded that ISI (ogtt) derived from their equation is more suitable than other for assessing insulin sensitivity in subjects with normal glucose tolerance.

Zofia Cervenakova *et al*.,(2002) done a study on **"Effect of body composition on indices of insulin sensitivity and beta-cell function in healthy men"** the aim of this study was to evaluate the influence of body composition on various indices of insulin sensitivity and secretion in subjects with normal glucose tolerance. A total of 33 male subjects (aged 26-51 years,BMI19.7-30.9kg/m^2) underwent a standard oral glucose tolerance tests and they were measured using indices of insulin sensitivity and the results showed that all subjects had a normal glucose tolerance no difference was found in course of glycemia, while over weight subjects had an enhanced insulin response. The indices of insulin sensitivity all significantly increased in overweight group. And finally they concluded this study is that easiest way of predicting insulin resistance in normal glucose tolerance is to calculate an index from glucose and insulin concentration during an ogtt.

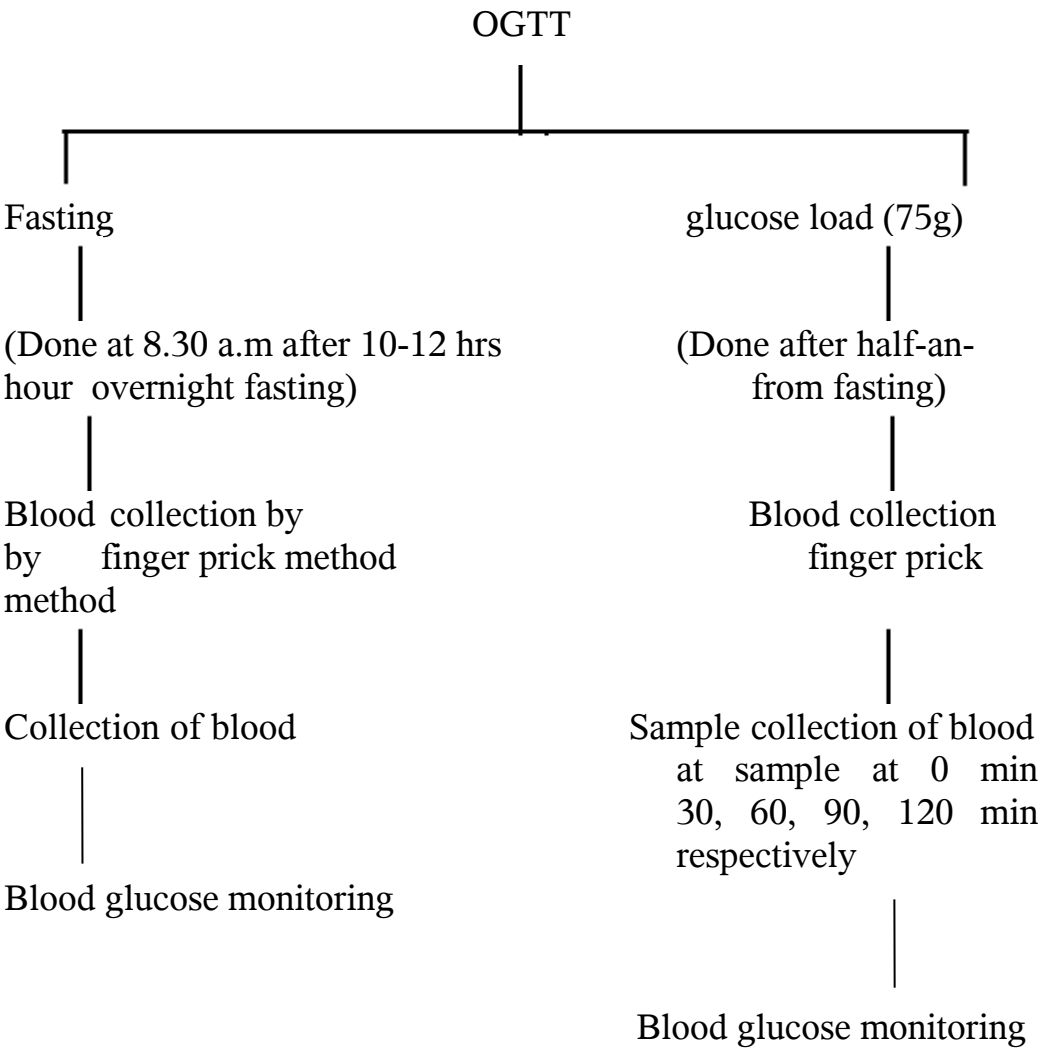
M.Albareda *et al.,* (2000) **"Assessment of insulin sensitivity and beta cell function from measurements in the fasting state and during an oral glucose tolerance test"** the aim of the study was to find if the relation between insulin sensitivity and beta cell function assessed from fasting and ogtt measurements has a physiological shape. The study was performed with healthy women without diabetic first degree relatives underwent a 75g ogtt with a

plasma glucose and insulin (n=35) concentration being measured at (0,30,60 and 120) beta cell function and insulin sensitivity were measured using indices. A hyperbolic relation was tested for 21 beta cell function insulin sensitivity pairs using a non-lineal regression method. Finally they conclude the study by the estimation of insulin sensitivity and beta cell function by most using method ogtt did not adjust to hyperbolic relation in healthy women but fasting indices combination did and beta cell function estimated the HOMA index and insulin sensitivity with fasting glucose to insulin ratio had the best adjustment.

Michael Stumvoll *et al.,* (2000) made a study on **"Use of oral glucose tolerance test to assess insulin release and insulin sensitivity "**the aim of this study was that the oral glucose tolerance test has been often been used to evaluate apparent insulin release and insulin resistance in variable clinical settings. They studied with non diabetic volunteers who had undergone ogtt and euglycemic hyperinsulinemic clamp for assessing insulin sensitivity and insulin release. Demographic parameters and plasma glucose and insulin values from ogtt were subjected to multiple linear regression to predict metabolic clearance rate of glucose, the ISI and Ist phase and 2^{nd} phase insulin release as measured with respective clamps. Finally they concluded this study that predicting insulin sensitivity and insulin release with reasonable accuracy from simple demographic parameters obtained during ogtt is possible.the derived equation would be impractical in various clinical setting.

D.Tripathy *et al.,* (2000) made a study on **"Insulin secretion and insulin sensitivity in diabetic subgroups: studies in the prediabetic and diabetic state"** the aim of this study was to evaluate insulin sensitivity and insulin secretion in prediabetic and diabetic subjects with mutation in mody1 and mody3 genes, in subjects with GAD antibodies ,latent autoimmune diabetes in adults and in subjects with the common form of type II diabetic mellitus. Insulin secretion was measured as the incremental 30min insulin and insulin glucose ratio during ogtt and also insulin sensitivity was measured as insulin sensitivity index in all types of diabetic patients and also these subgroup subjects underwent a euglycemic clamp intravenous glucose tolerance test for estimation of insulin sensitivity and first phase insulin secretion. Finally they concluded the study by that glucose tolerance carriers of mody mutation are characterized by severe impairment in insulin secretion. Enhanced insulin sensitivity seen in normal glucose tolerance. In subjects with positive GADA or type2 have impaired insulin sensitivity. mody mutation carriers were protected from the effect of glucose toxicity.

METHODOLOGY



PRINCIPLE FOR INSULIN ASSAY

The Insulin Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-Insulin antibody for solid phase (microtiter wells) immobilization and another anti-Insulin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The standards and test specimen (serum) are added to the Insulin antibody coated microtiter wells. Then anti- Insulin antibody labeled with horseradish peroxidase (conjugate) is added. If human Insulin is present in the specimen, it will combine with the antibody on the well and the enzyme conjugate resulting in the Insulin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 1 hour incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of chemiluminescent substrate is then added and read relative light units (RLU) in a Lumino meters. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of INSULIN in the sample. By reference to a series of INSULIN standards assayed in the same way, the concentration of INSULIN in the unknown sample is quantified.

MATERIALS

- Xylene-manufactured by fischer chemic ltd.,
- Sterile Absorbent Cotton-manufactured by The Ramaraju Surgical Cotton Mills Ltd.,
- Sterile blood lancets-manufactured by Medipoint, Inc.,
- Blood glucose test strips- manufactured by Major Biosystem Corp.
- Glucose-D-(1 kg)-manufactured by Avalon Cosm. Pvt. Ltd.,
- Centrifuge tubes (1 ml)- Eppendorf Ltd.,
- Disposable syringe (5 ml)-BD
- Vaccum blood collection tube (5ml)- manufactured by peerless biotech private ltd.,
- Disposable filler-BD
- Precision pipettes and tips, 0.05 ml, 0.1 ml – Tarsons Products Pvt. Ltd.,
- Disposable pipette tips- Himedia
- Distilled water
- Absorbent paper
- Microtiter plate reader- Tarsons Products Pvt. Ltd.,
- Monoclonal anti Insulin antibody coated microtiter plate with 96 wells.
- Enzyme conjugate reagent, 12 ml.
- Insulin reference standards containing; 0, 5, 25, 50, 100, and 200 uIU/ml. lyophilized 0.5mlx2sets.

- Wash Solution Concentrate, 50X, 15ml
- Chemiluminescence Reagent A, 6.0 ml.
- Chemiluminescence Reagent B, 6.0 ml.

REAGENTS FOR INSULIN ASSAY

1. All reagents were kept at room temperature (18-25°C) and mixed gently by inverting or swirling without formation of foam.
2. 1 volume of Wash Buffer (50x) was diluted with 49 volumes of distilled water
3. Each lyophilized standard was reconstituted with 0.5 ml of distilled water and was allowed to stand for 20 minutes.

INSTRUMENT:

OGTT	-	Gluco Chek blood glucose monitoring system- manufactured by Major Biosystem Corp Taiwan
INSULIN ASSAY	-	Immulite 2000
Vortex mixer	-	Remi Motors Ltd.,
Centrifuge	-	Eppendorf Ltd.,

METHODOLOGY

I. OGTT

Currently two methods are available:

a. Traditional Method- Finger Prick Method

b. Glucose Oxidase Method

a. TRADITIONAL METHOD

Blood glucose meter is a small portable machine used to monitor blood glucose levels.

PROCEDURE

1. Hands were washed in warm, soapy water and dried thoroughly.
2. Finger tip was cleansed using sterile cotton soaked in spirit
3. The finger was then allowed to dry.
4. The test strip was inserted into the slot of the glucose meter with the black bars of the test strip facing up.
5. Fingertip was pricked using a sterile lancet and gently squeezed to get a drop of blood.
6. The blood drop was placed on the test strip, previously inserted into the glucose meter and monitored for blood glucose levels.
7. The glucose meter soon displays the blood glucose level as a number in mg/dl unit.

INSULIN ASSAY

PROCEDURE FOR SERUM COLLECTION

1. A tourniquet was placed around the upper arm to apply pressure and cause the veins to swell with blood.
2. Blood was drawn from a vein in the arm inside of the elbow after cleaning the skin surface with an antiseptic and collected in a syringe.
3. The blood was then collected in a plain red-top venipuncture tube without additives.
4. The blood was then allowed to clot.
5. The specimen was then centrifuged to separate the serum from cells.

INSULIN ASSAY PROCEDURE

1. Desired number of coated wells was secured in the holder. 50µl of Insulin standards, specimens, and controls were added into the appropriate wells and mixed gently for 10 seconds.
2. 100µl of enzyme conjugate reagent was added into each well and mixed gently for 30 seconds to facilitate complete mixing and incubated at room temperature for 60 minutes.
3. The incubation mixture was then removed and the microtiter plate was rinsed 5 times with 1 x wash buffer (300µl each well). Then the residual water droplets were removed using absorbent paper.
4. 100 µl Chemiluminescence substrate solution was then added into each well and gently mixed for 5 seconds.

5. After 5 minutes, the wells were observed using a chemiluminescence microwell reader.

Methodology for AUC calculation

Total AUCs for glucose and insulin were calculated using the trapezoidal rule.

Methodology for cluster analysis

Data often fall naturally into groups / clusters, of observations, where the characteristics of objects in the same cluster are similar. K-means clustering (partitioning) treats observations in the data as objects having locations and distances from each other. It partitions the objects into K mutually exclusive clusters, such that objects within each cluster are as close to each other as possible, and as far from objects in other clusters as possible. Each cluster is characterized by its centroid (center point). Of course, the distances used in clustering often do not represent spatial distances. Hierarchical clustering investigates grouping in the data, simultaneously over a variety of scales of distance, by creating a cluster tree. The tree is not a single set of clusters, as in K-Means, but rather a multi-level hierarchy, where clusters at one level are joined as clusters at the next higher level. This allows us to decide what scale or level of clustering is most appropriate in our application.

Variables were analyzed by a computer program which permits direct visualization of the three dimensional shape of the data set and the subjects were classified by means of a computer

classification which employed a cluster analysis technique. This resulted in the definition of three groups.

Methodology for calculating HOMA-IR

Homeostasis model assessments of insulin resistance (HOMA2-IR) and pancreatic beta-cell function (HOMA2-%B) were completed using the HOMA Calculator version 2.2.2 (**http://www.dtu.ox.ac.uk, accessed Feb 2010).**

INDICES CALCULATION

The indices evaluated were selected *a priori* based on their performance in previous investigations.The **insulin sensitivity index** (ISI) was calculated from the oral glucose tolerance test according to the formula:

ISI = 10,000 ÷ √ ([fasting plasma glucose × fasting plasma insulin] × [mean OGTT glucose × mean OGTT insulin]).

Beta-cell function was assessed as corrected incremental insulin response (CIR) during the glucose-tolerance test according to the formula:

CIR = (100 × insulin at 30 min) ÷ ([glucose at 30 min] × [glucose at 30 min − 3.89])

(or) as a disposition index (i.e., insulin secretion adjusted for insulin sensitivity, or CIR × ISI).

RESULTS

Table 8: OGTT Glucose Concentrations in mg/dL

Status	code	0min	30min	60min	90min	120min
FH	122	78	159	178	148	125
FH	121	75	161	176	148	129
FH	171	81	166	176	119	70
FH	172	83	151	175	119	75
FH	71	104	118	159	116	103
FH	72	105	133	154	138	89
FH	181	103	156	147	110	98
FH	21	98	110	140	129	88
	131	81	115	139	126	79
	14	**65**	**133**	**134**	**109**	**74**
	19	**98**	**147**	**132**	**114**	**100**
	52	94	137	124	104	95
	1	77	133	124	93	82
	16	109	137	123	109	98
	4	87	147	122	111	84
	10	91	131	119	96	105
	9	81	141	118	96	85
	15	88	131	117	102	89
	82	94	156	115	100	93
	132	87	128	115	97	107
	3	88	131	115	91	84
	20	80	137	114	105	96
	11	97	136	113	100	68
	62	90	133	112	70	93
	2	***59***	***135***	***111***	***86***	***77***
	182	101	133	111	111	98
	61	93	104	98	60	94
	51	94	131	84	75	83
	81	91	96	68	70	89

Fig1: OGTT CURVE

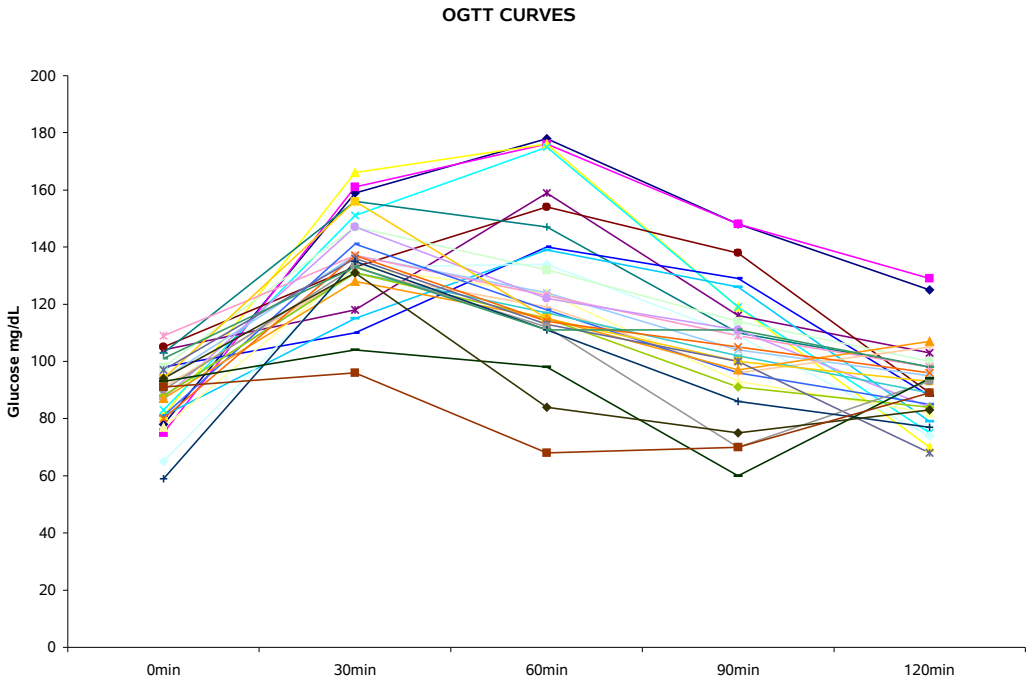


Fig 2: OGTT curve for Subjects without Family history of Diabetes

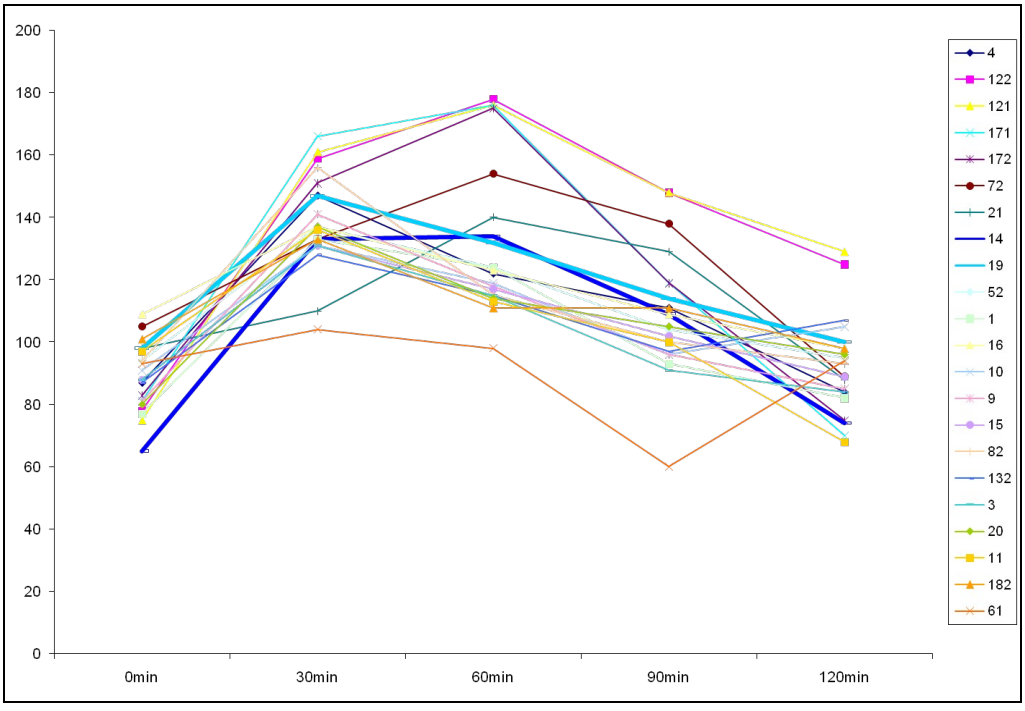


Fig 3: OGTT (NGR group) -3rd order polynomial fit

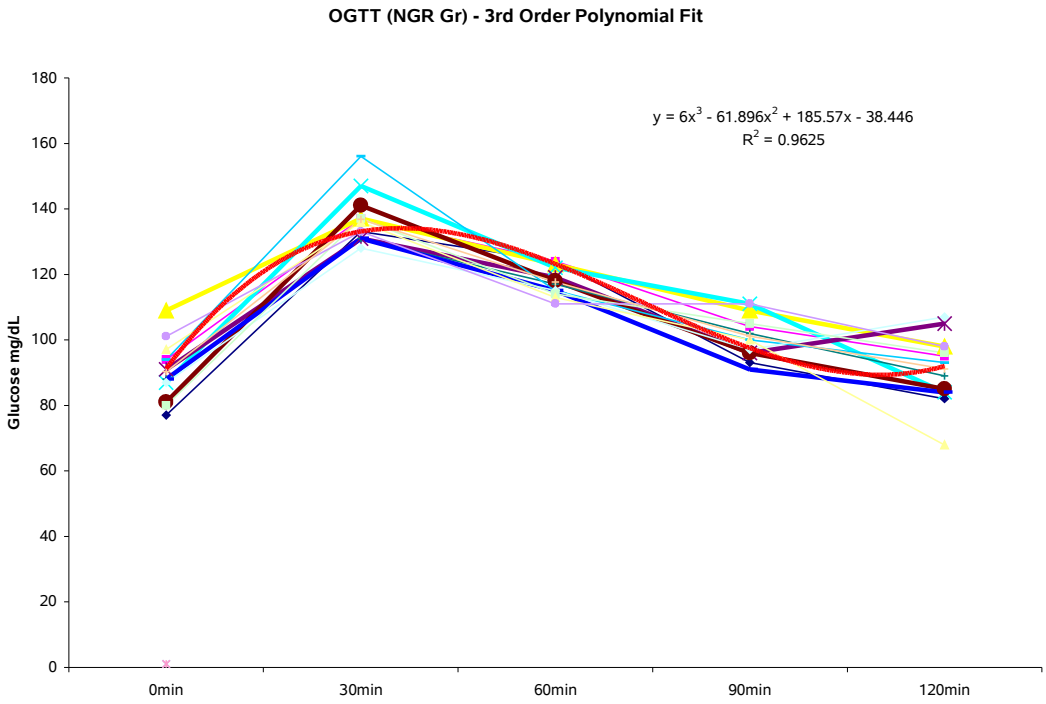


Fig 4 :OGTT (IGR group)-3rd order polynomial fit

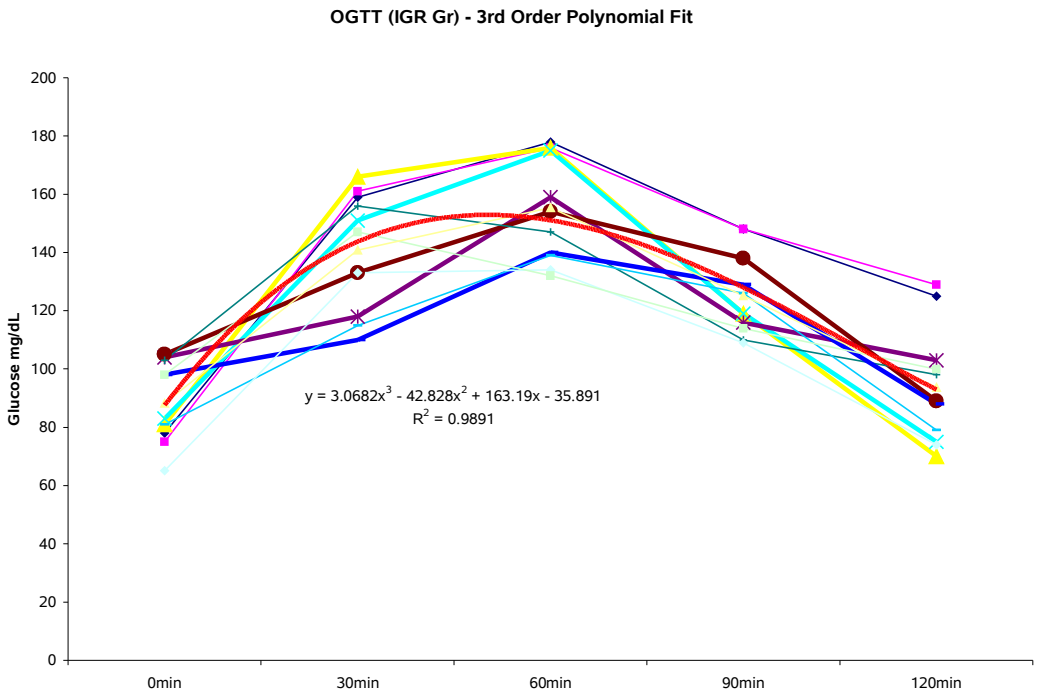


Fig 5: OGTT insulin group

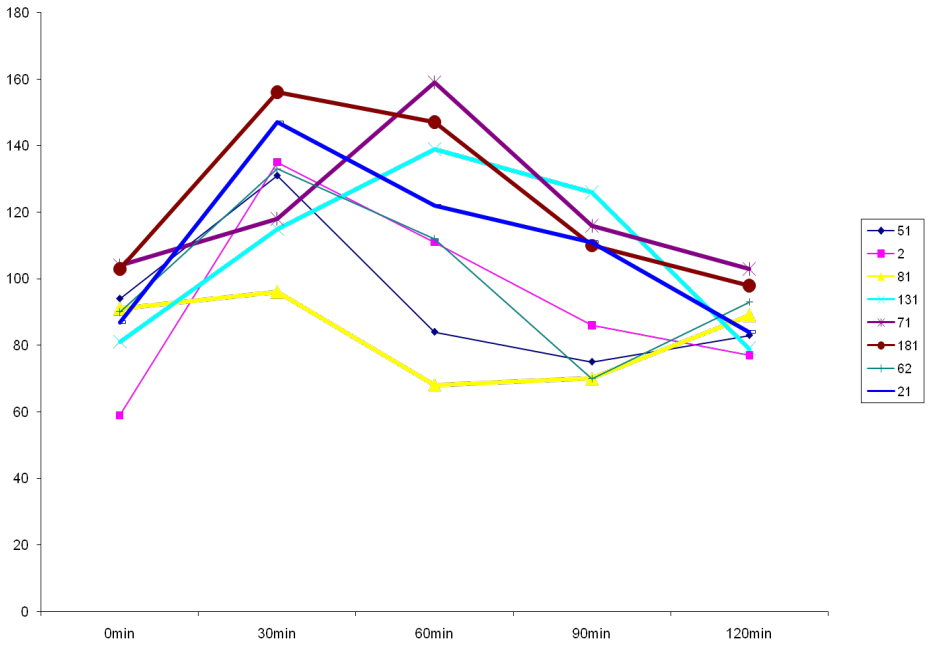
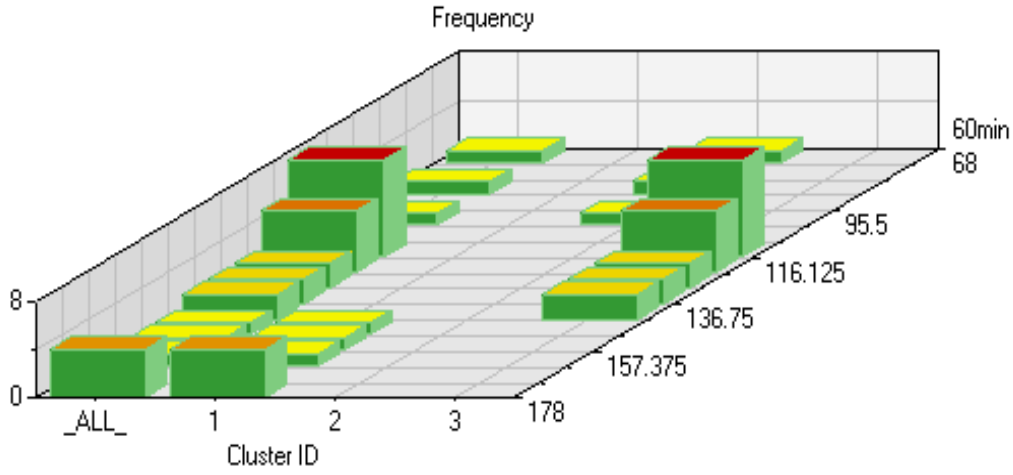


Fig6: Cluster analysis of OGTT blood glucose concentrations



Cluster	Frequency
1	7
2	3
3	19

Variable	Total std	Within std	R-square	Rsq/ (1-rsq)
30min	16.48899	12.98106	0.424497	0.737609
60min	26.87602	10.66505	**0.853778**	**5.838931**
90min	21.45370	13.79622	0.616000	1.604167
OVER-ALL	22.01869	12.55106	**0.698288**	**2.314416**

Table 9: Cluster Analysis for All Subjects

OBS	CODE	CLUSTER
1	1	3
2	2	3
3	3	3
4	4	3
5	**51**	**2**
6	52	3
7	**61**	**2**
8	62	3
9	**71**	**1**
10	**72**	**1**
11	**81**	**2**

12	82	3
13	9	3
14	10	3
15	11	3
16	121	1
17	122	1
18	131	3
19	132	3
20	14	3
21	15	3
22	16	3
23	171	1
24	172	1
25	181	1
26	182	3
27	19	3
28	20	3
29	21	3

Fig 7: OGTT -60min curve for all subjects

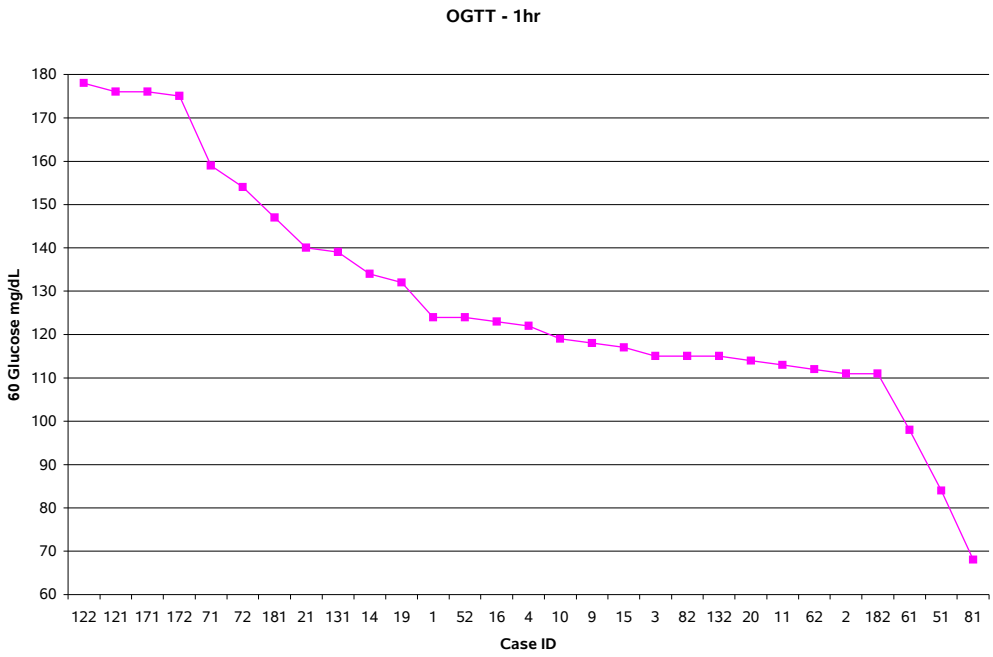


Fig 8: OGTT - AUCg for all subjects

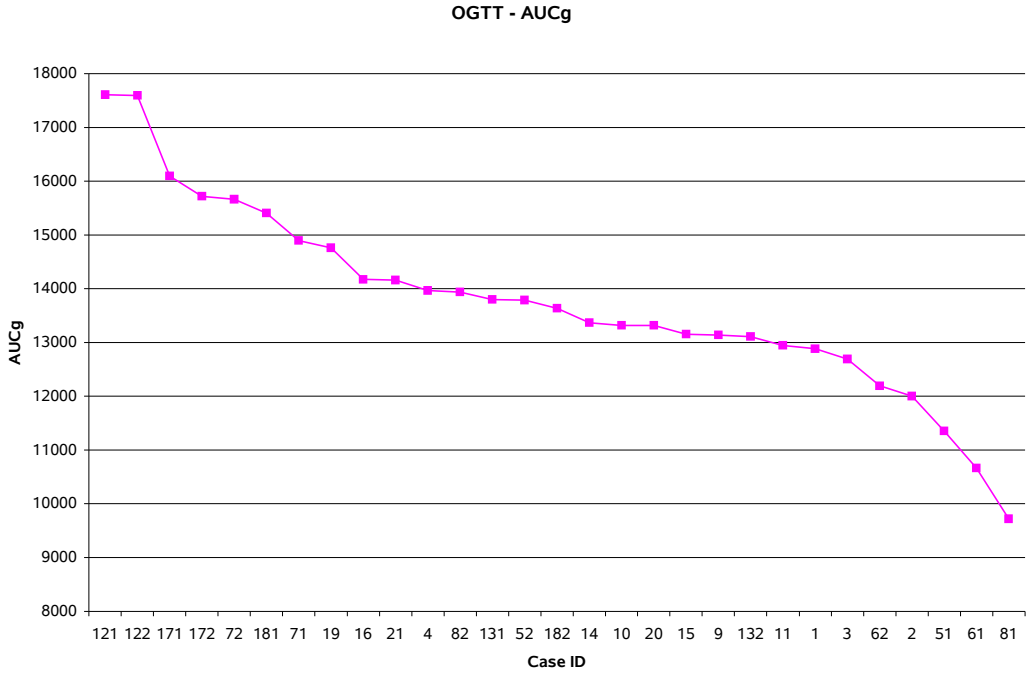


Fig :9 Correlation of AUCg with 1hr OGTT glucose

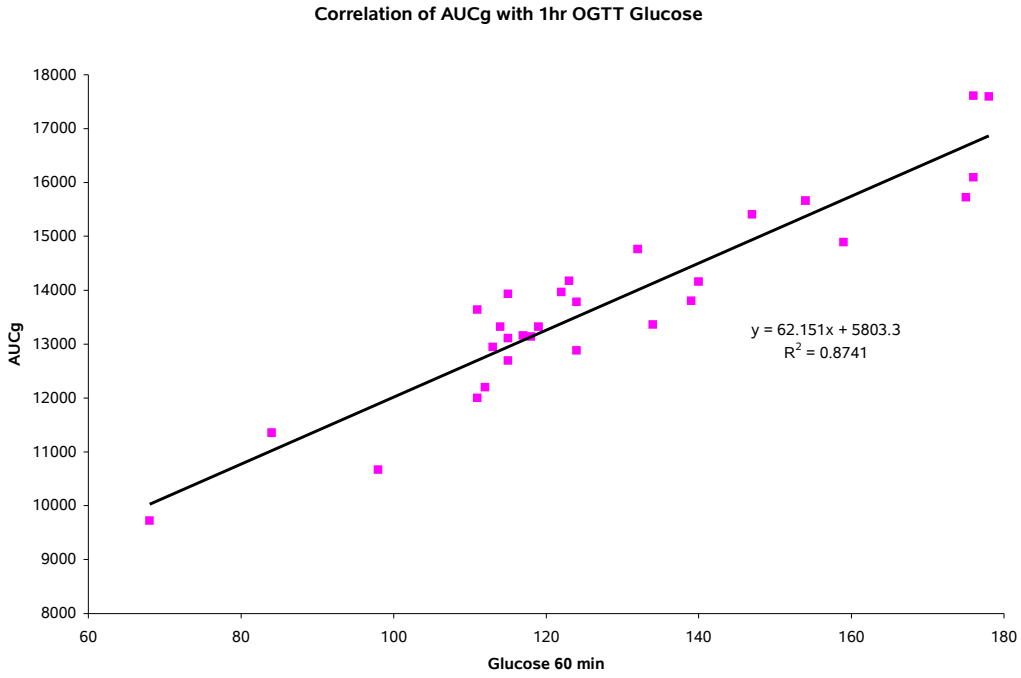


Fig:10 Correlation of AUCg with 1hr OGTT glucose (NGR group)

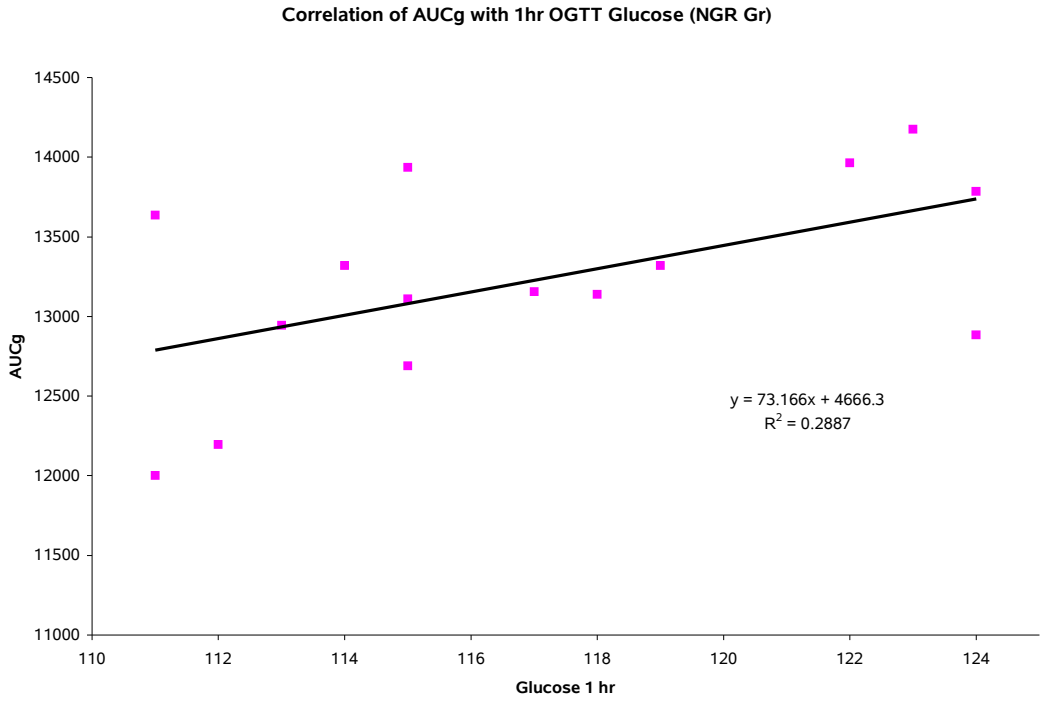


Fig:11 Correlation of AUCg with 1hr OGTT glucose (IGR group)

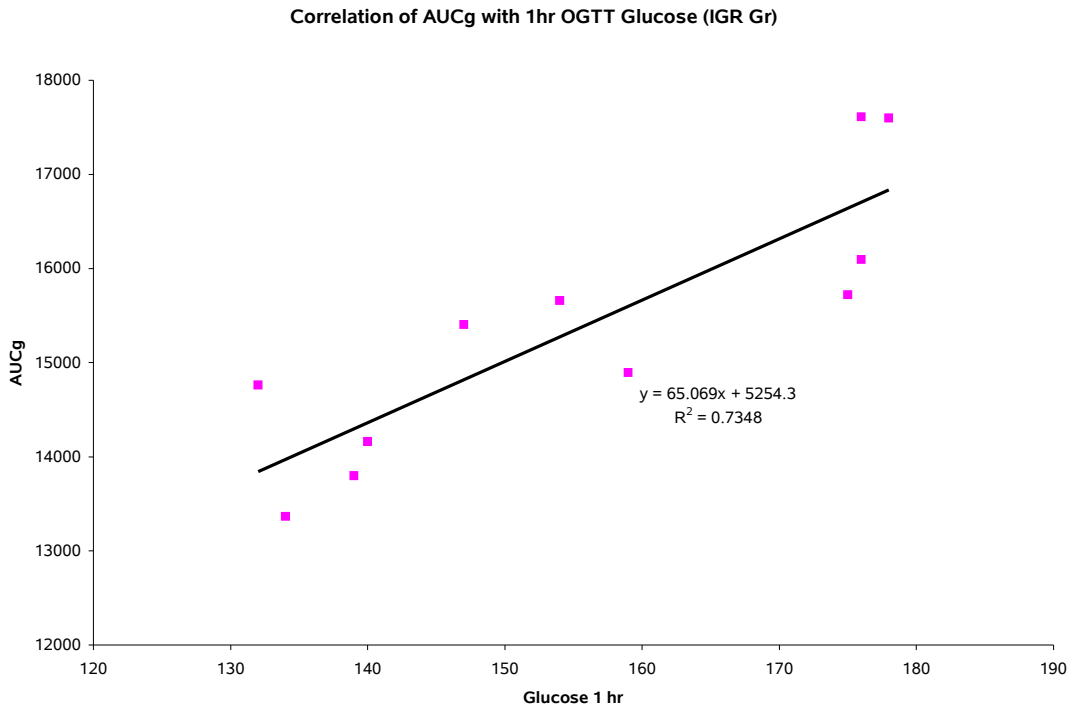


Table 10: AUCg Calculations for all the subjects

Case ID	Time Interval	First	Last	Minimum	Maximum	AUC (baseline = 0)
1	120	77	82	77	133	12885.0000
2	120	59	77	59	135	12000.0000
3	120	88	84	84	131	12690.0000
4	120	87	84	84	147	13965.0000
51	120	94	83	75	131	11355.0000
52	120	94	95	94	137	13785.0000
61	120	93	94	60	104	10665.0000
62	120	90	93	70	133	12195.0000
71	120	104	103	103	159	14895.0000
72	120	105	89	89	154	15660.0000
81	120	91	89	68	96	9720.0000
82	120	94	93	93	156	13935.0000
9	120	81	85	81	141	13140.0000
10	120	91	105	91	131	13320.0000
11	120	97	68	68	136	12945.0000
121	120	75	129	75	176	17610.0000
122	120	78	125	78	178	17595.0000
131	120	81	79	79	139	13800.0000
132	120	87	107	87	128	13110.0000

14	120	65	74	65	134	13365.0000
15	120	88	89	88	131	13155.0000
16	120	109	98	98	137	14175.0000
171	120	81	70	70	176	16095.0000
172	120	83	75	75	175	15720.0000
181	120	103	98	98	156	15405.0000
182	120	101	98	98	133	13635.0000
19	120	98	100	98	147	14760.0000
20	120	80	96	80	137	13320.0000
21	120	98	88	88	140	14160.0000

Group	n	AUC Mean	95% CI	SD	AUC Median	95% CI
(All cases)	29	13760.690	13081.109 To 14440.271	1786.586	13635.000	13132.978 to 14163.511

Table 11: Cluster analysis of AUCg Values

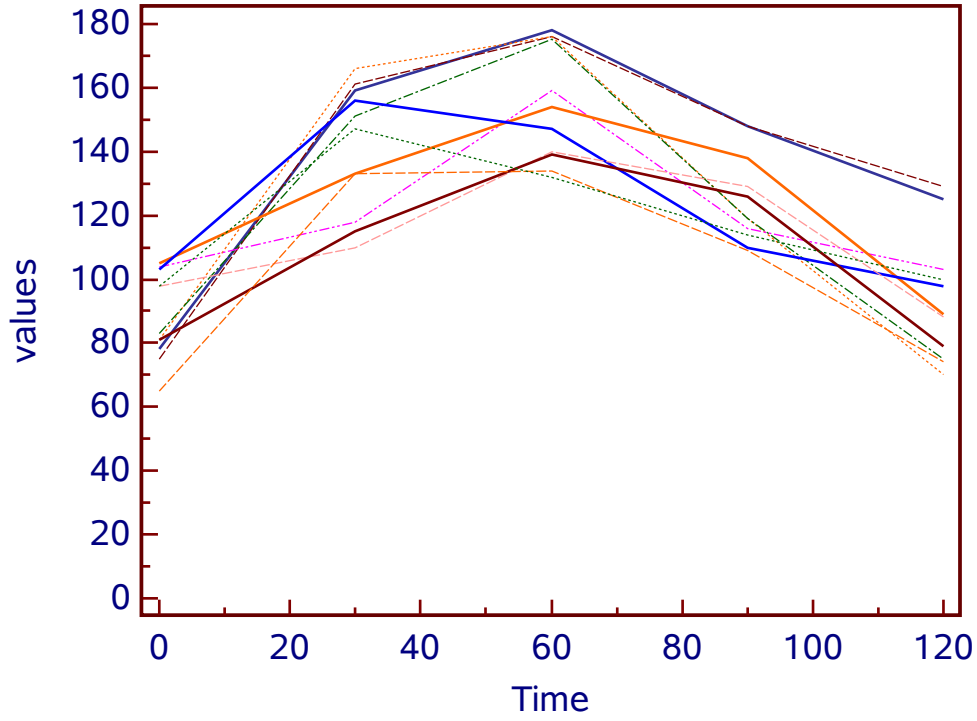
OBS	CASE ID	CLUSTER
1	**121**	**1**
2	**122**	**1**
3	**171**	**1**
4	172	3
5	72	3
6	181	3
7	71	3
8	19	3
9	16	3
10	21	3
11	4	3
12	82	3
13	131	3
14	52	3
15	182	3
16	14	3
17	10	3
18	20	3

19	15	3
20	9	3
21	132	3
22	11	3
23	1	3
24	**3**	**2**
25	**62**	**2**
26	**2**	**2**
27	**51**	**2**
28	**61**	**2**
29	**81**	**2**

Cluster	Frequency
1	3
2	6
3	20

Variable	Total std	Within std	R-Square	RSQ/(1-RSQ)
AUC	1787	935.78127	**0.745249**	2.925398
OVER-ALL	1787	935.78127	0.745249	2.925398

Fig:12. AUCg plot for IGR group



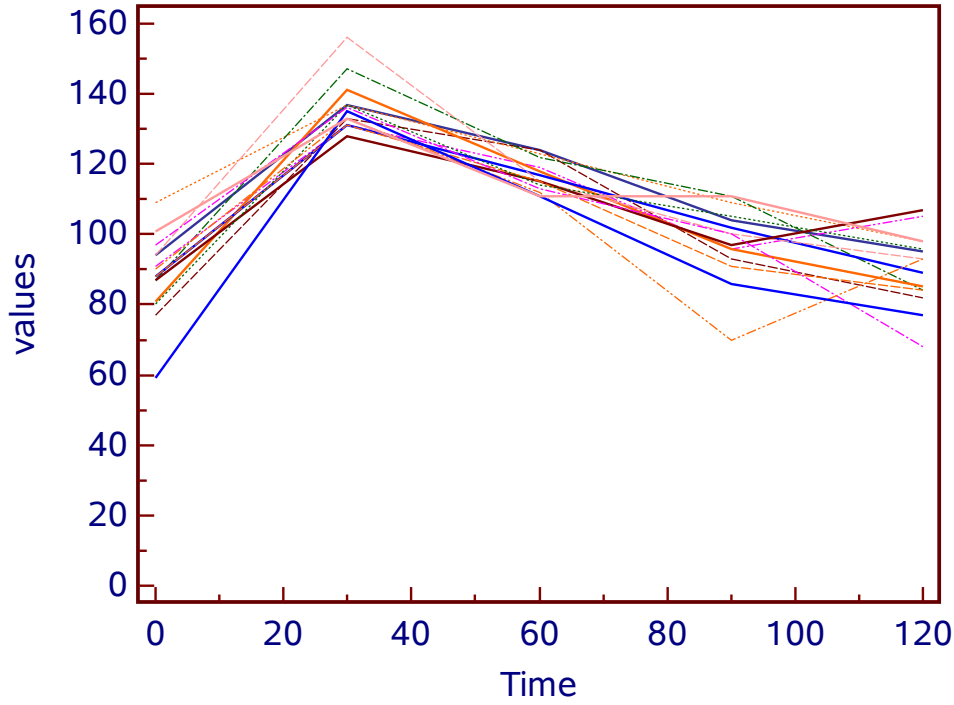
Group	n	Mean	95% CI	SD	Median	95% CI
(All cases)	11	15369.545	14437.486 to 16301.605	1387.387	15405.000	14095.310 to 16364.540

Table 12: AUCg calculation for IGR group

Case ID	Time Interval	First	Last	Minimum	Maximum	AUC (baseline = 0)
122	120	78	125	78	178	17595.0000
121	120	75	129	75	176	17610.0000
171	120	81	70	70	176	16095.0000
172	120	83	75	75	175	15720.0000
71	120	104	103	103	159	14895.0000
72	120	105	89	89	154	15660.0000
181	120	103	98	98	156	15405.0000
21	120	98	88	88	140	14160.0000
131	120	81	79	79	139	13800.0000
14	120	65	74	65	134	13365.0000

19	120	98	100	98	147	14760.0000

Fig:13. AUCg plot for NGR group



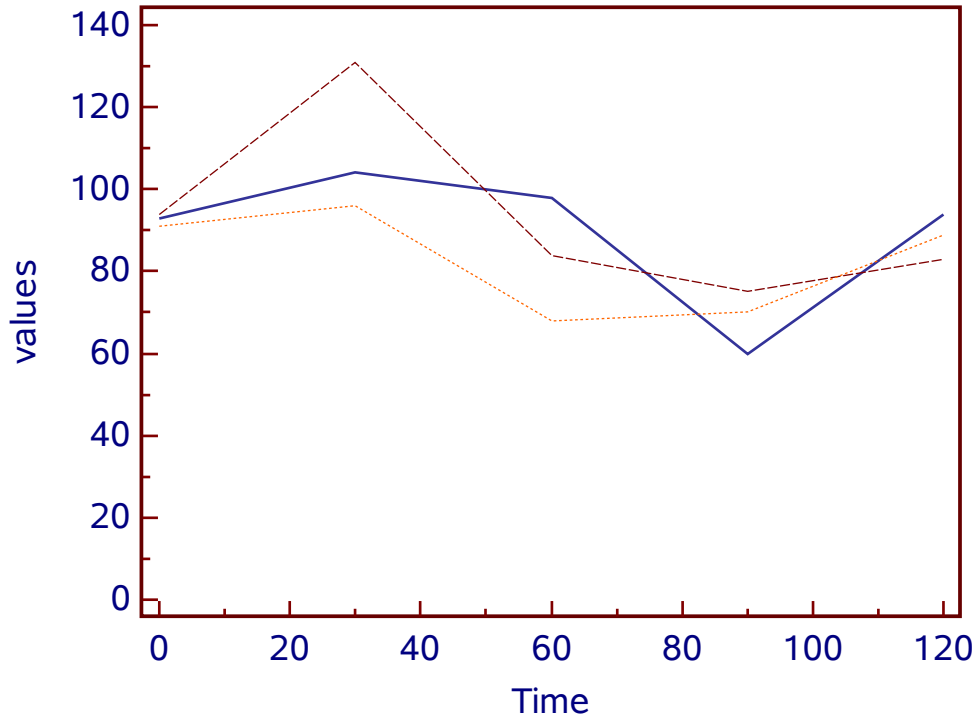
Group	n	Mean	95% CI	SD	Median	95% CI
(All cases)	15	13217.000	12869.266 to 13564.734	627.927	13155.000	12900.895 to 13745.262

Table 13: AUCg calculation for NGR group

Case ID	Time Interval	First	Last	Minimum	Maximum	AUC (baseline = 0)
52	120	94	95	94	137	13785.0000
1	120	77	82	77	133	12885.0000
16	120	109	98	98	137	14175.0000
4	120	87	84	84	147	13965.0000
10	120	91	105	91	131	13320.0000
9	120	81	85	81	141	13140.0000
15	120	88	89	88	131	13155.0000

82	120	94	93	93	156	13935.0000
132	120	87	107	87	128	13110.0000
3	120	88	84	84	131	12690.0000
20	120	80	96	80	137	13320.0000
11	120	97	68	68	136	12945.0000
62	120	90	93	70	133	12195.0000
2	120	59	77	59	135	12000.0000
182	120	101	98	98	133	13635.0000

Fig 14: AUCg plot for Low Sugar Group



Group	n	Mean	95% CI	SD	Median	95% CI
(All cases)	3	10580.000	8541.001 to 12618.999	820.808	10665.000	

Table 14: AUCg calculation for low sugar group

Case ID	Time Interval	First	Last	Minimum	Maximum	Area under curve (baseline = 0)
61	120	93	94	60	104	10665.0000
51	120	94	83	75	131	11355.0000
81	120	91	89	68	96	9720.0000

Table 15: OGTT Insulin values for selected individuals

Status	Code	Ins 0MIN	Ins 30MIN	Ins 120MIN	0min	30min	60min	90min	120min
	4	6.8	4.4	1	87	147	122	111	84
	2	*6.4*	*53.6*	*12.5*	*59*	*135*	*111*	*86*	*77*
	51	3.7	66.7	2.2	94	131	84	75	83
	62	3.1	17.2	2.8	90	133	112	70	93
	81		53.4		91	96	68	70	89
	131		52.6		81	115	139	126	79
FH	71		33.2		104	118	159	116	103
FH	181		29		103	156	147	110	98

Fig15. OGTT Insulin Plot for selected individuals

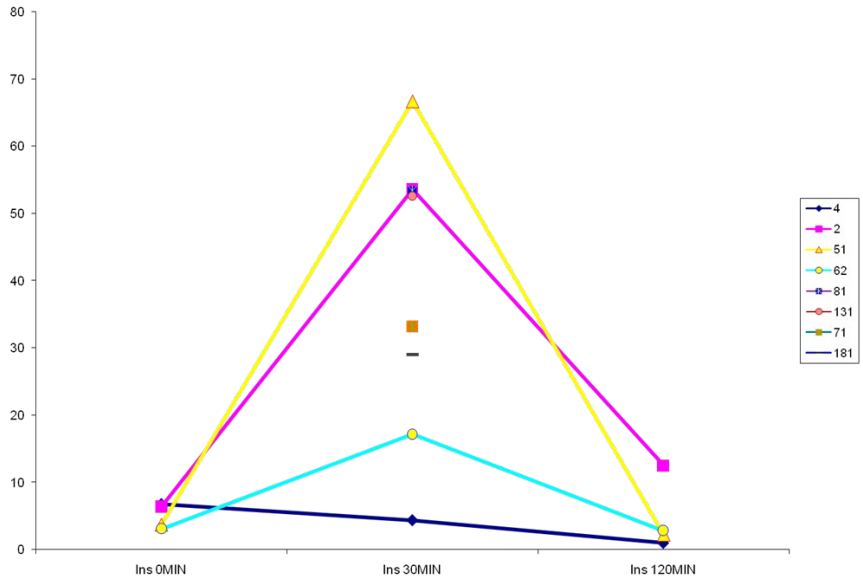
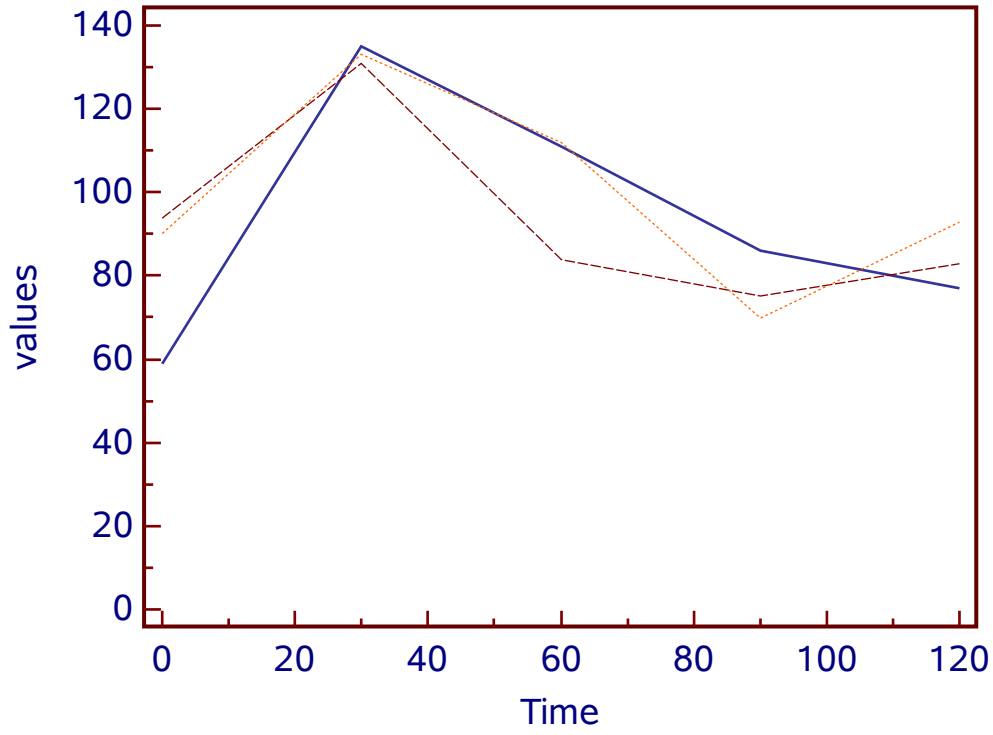


Fig:16. AUCg plot for selected individuals

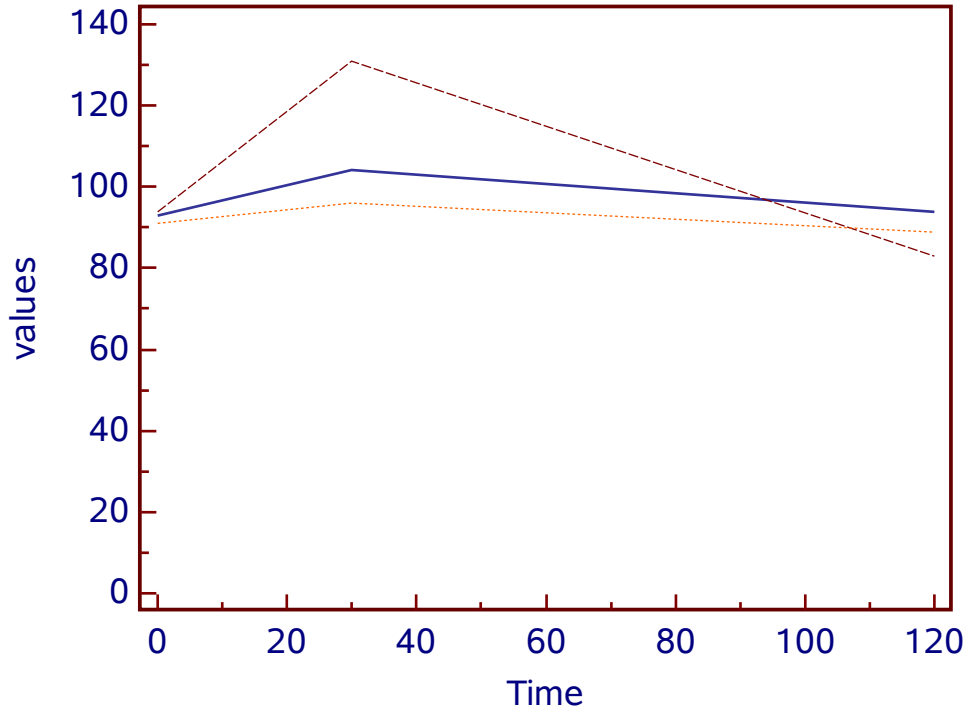


Group	n	Mean	95% CI	SD	Median	95% CI
(All cases)	3	11850.000	10757.897 to 12942.103	439.631	12000.000	

Table 16: AUCg calculation for OGTT insulin selected individuals

Case ID	Time Interval	First	Last	Minimum	Maximum	AUC Glucose (baseline = 0)
2	120	59	77	59	135	12000.0000
51	120	94	83	75	131	11355.0000
62	120	90	93	70	133	12195.0000

Fig:17. AUC Insulin for selected individuals



Group	n	Mean	95% CI	SD	Median	95% CI
(All cases)	3	12000.000	9653.081 to 14346.919	944.762	11865.000	

Table 17:
AUC insulin calculation

Case ID	Time Interval	First	Last	Minimum	Maximum	AUC Insulin (baseline = 0)
2	120	93	94	93	104	11865.0000
51	120	94	83	83	131	13005.0000
62	120	91	89	89	96	11130.0000

Fig:18. Clustering with 30 min Insulin & 60 min Glucose

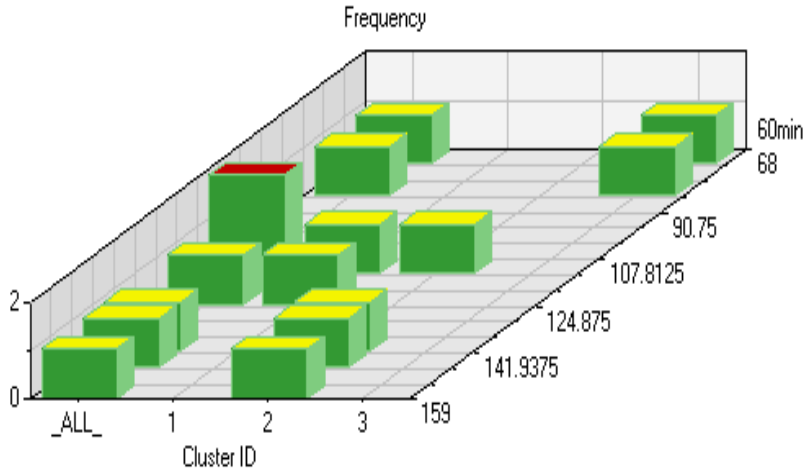


Table:18 Cluster analysis for insulin performed subjects

OBS	code	CLUSTER
1	4	1
2	2	2
3	51	3
4	62	1
5	81	3
6	131	2
7	71	2
8	181	2

Cluster	Frequency
1	2
2	4
3	2

Variable	Total std	Within Std	R-Square	RSQ/ (1-RSQ)
INS 30 MIN	21.30848	11.52115	0.791186	3.788957
GLU 60 MIN	30.98271	16.88787	0.7877782	3.712132
OVER ALL	26.58928	14.45574	0.78875	3.736531

Table:19 Clustering ONLY WITH GLUCOSE 60 MINUTES

Cluster	Frequency
1	3
2	2
3	3

Variable	Total std	Within std	R-square	RSQ/ (1-RSQ)
GLU 60 MIN	30.98271	8.99630	**0.939777**	15.605025
OVER ALL	30.98271	8.99630	0.939777	15.605025

Fig: 19. 30MIN INSULIN VS 30 MIN GLUCOSE

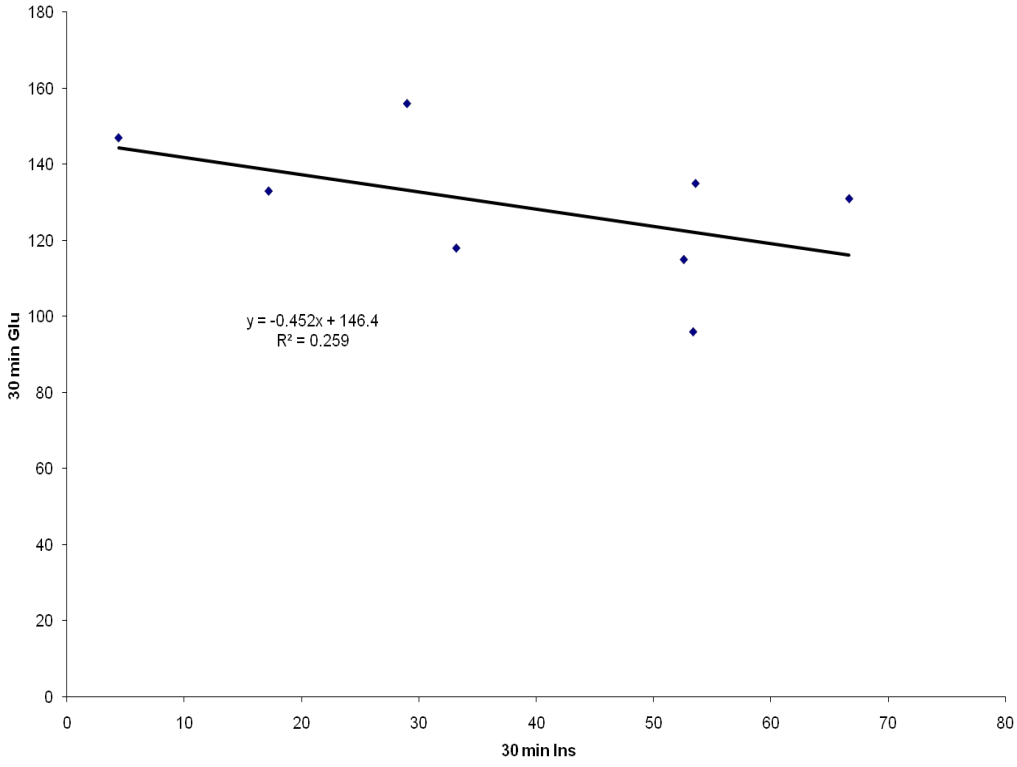


Fig:20. 30 MINUTES INSULIN VS 60MINUTES GLUCOSE:

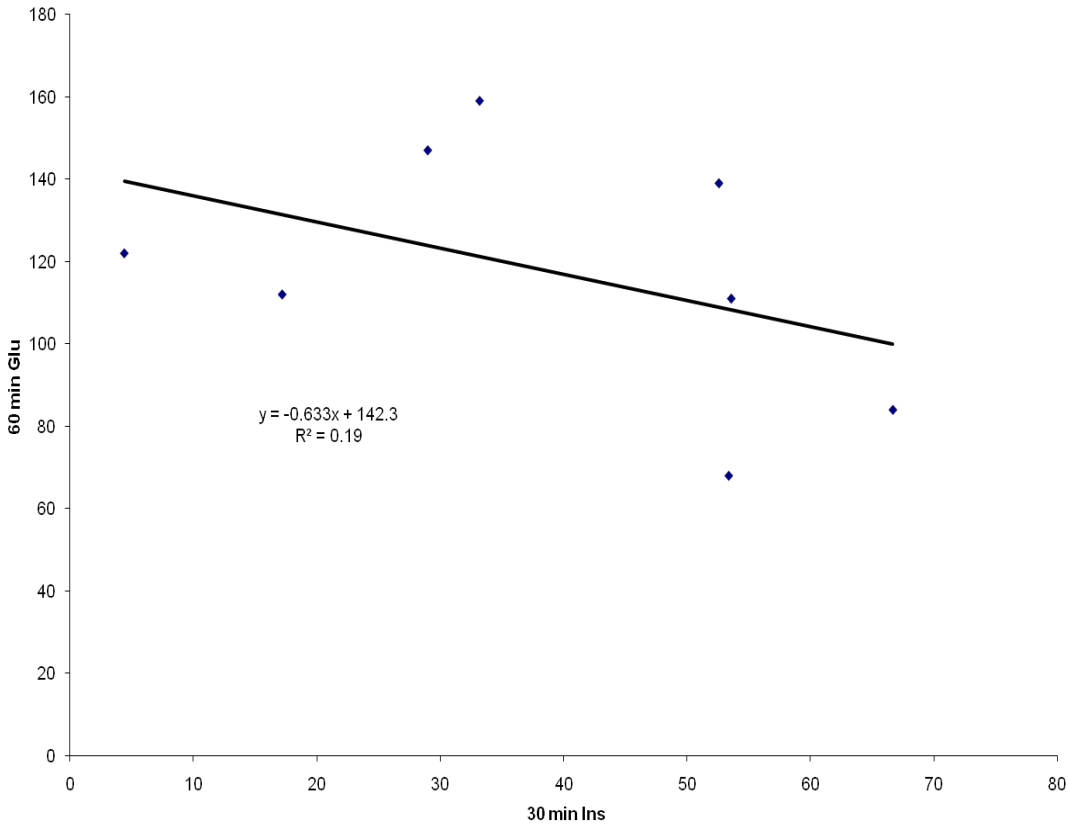


Table 20: Indices Calculation

CODE	Glucose 0min	Insulin 0min	HOMA2 %B	HOMA2 %S	HOMA2 IR
2	***59***	6.4	197.6	136.0	0.7
51	**94**	**3.7**	53.3	204.2	0.5
62	**90**	3.1	51.8	246.2	0.4

Table 21: Insulin 30 min Group - Indices

Code	Glucose 30min	Insulin 30min	HOMA2 %B	HOMA2 %S	HOMA2 IR
2	135	53.6	175.5	14.2	7.0
51	**131**	**66.7**	-	-	-
62	133	17.2	78.2	41.6	2.4
81	**96**	53.4	316.4	15.4	6.5
131	**115**	**52.6**	**227.1**	**15**	**6.7**
71	**118**	**33.2**	**156.1**	**22.8**	**4.4**
181	**156**	**29**	**86.6**	**24.4**	**4.1**

Fig:21. Hyperbolic Curve Relationship

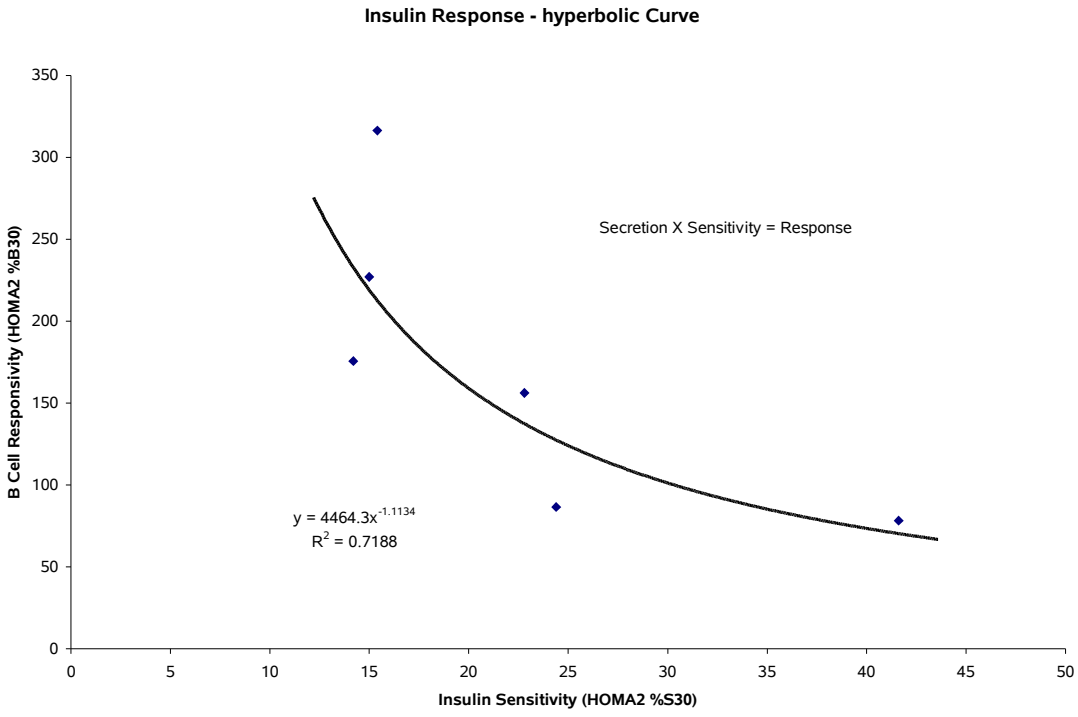


Fig:22. Early Phase Insulin Secretion

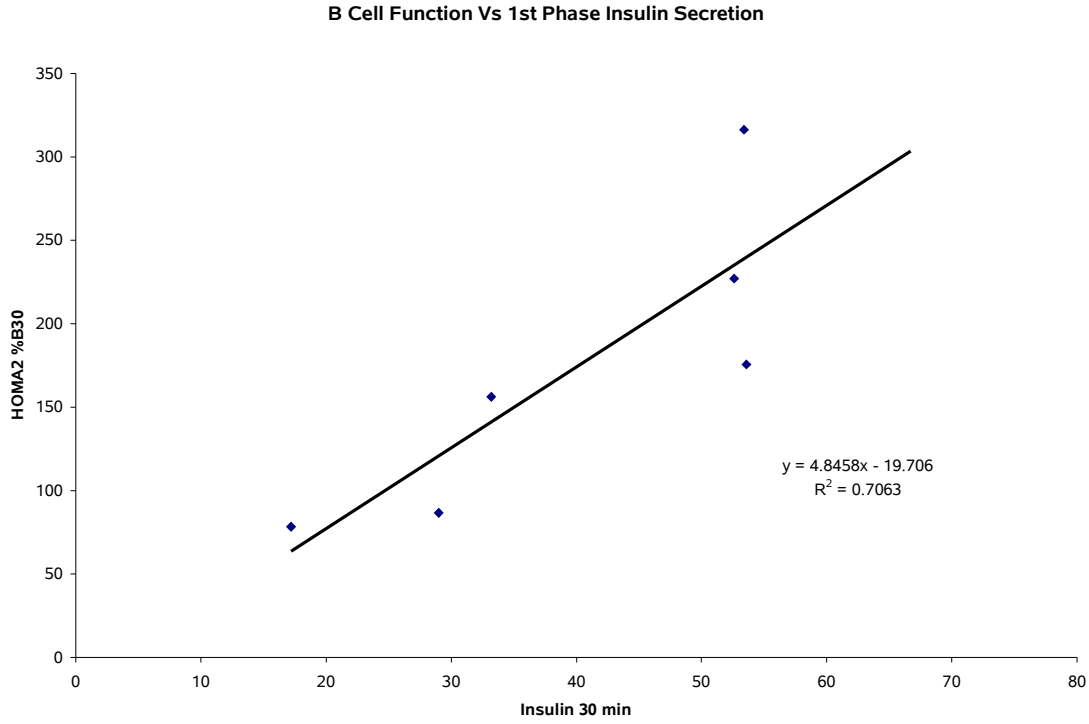
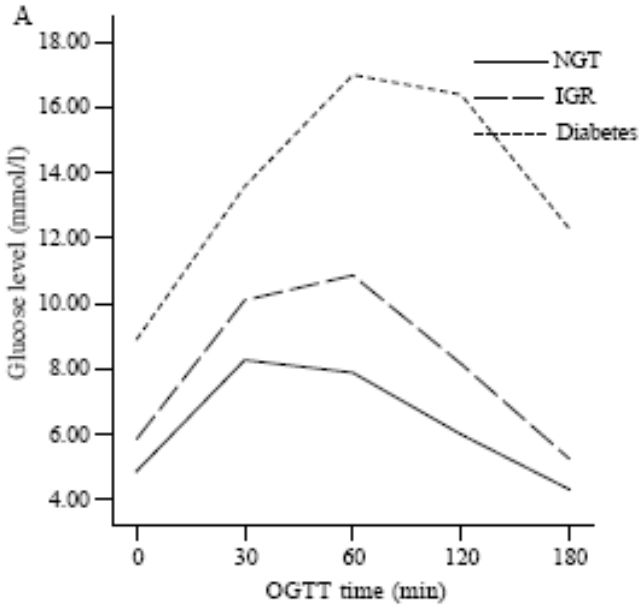


Fig:23 & 24. OGTT Curves Showing high glucose values at 60min discriminating pre-diabetes subtypes


A
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
Glucose level (mmol/l)
NGT
IGR
Diabetes
0
30
60
120
180
OGTT time (min)

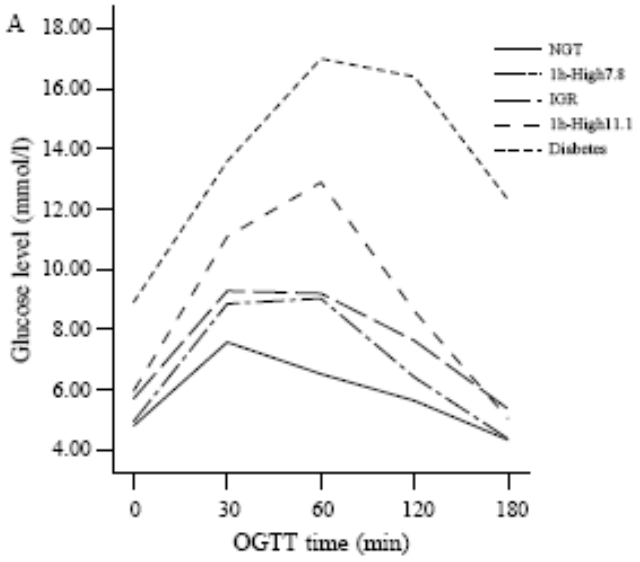

A
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
Glucose level (mmol/l)
NGT
1h-High7.8
IGR
1h-High11.1
Diabetes
0
30
60
120
180
OGTT time (min)

Table 22: Comparision of different cluster analysis routines

S. No	Level	Subjects (AUC_g)	Subjects (G_{60})	Cluster (Cluster)
1	Very High	121, 122	121, 122, 171, 172	
2	High	171, 172, 71, 72, 181, 19	71, 72, 181, 21, 131, 14, 19	121, 122, 71
3	Normal	16, 21, 4, 82, 131, 52, 182, 14, 10, 20, 15, 9, 132, 11, 1, 3, 62, 2	16, 4, 82, 52, 182, 10, 20, 15, 9, 132, 11, 1,3, 62, 2	171, 172, 16, 21, 4, 82, 131, 52, 182, 14, 10, 20, 15, 9, 132, 11, 1, 181, 131, 19
4	Low	51, 61, 81	51, 61, 81	3, 62, 2, 51, 61, 81

Table 23: Comparision of different cluster analysis routines

S. No	Level	n	Mean	Cut off (AUCg)
1	High (IGR)	11	15369.545	>16000
2	Normal	15	13217	14000 - 16000
3	Low	3	10580	<12000

DISCUSSION

We carried out oral glucose tolerance test (OGTT-glucose / insulin) with 29 healthy female individuals for exploring parameters associated with impaired glucose regulation. After over night fast, blood samples for determinations of capillary blood glucose and venous blood insulin concentrations are taken at 0, 30,60 and 120mins following a standard oral glucose load (75 g). The observations were then plotted (Table:8). The differences in the shape of the OGTT curves were observed (Fig: 1). OGTT Curves of individuals were plotted for comparision of variability in blood glucose concentrations (Fig:2).

Variations in glucose concentrations at time points 30 min, 60 min (max) and 90 min were recorded (Fig:1,4&5). The fasting and 2-hr glucose concentrations for all the subjects did not differ significantly. The shape/pattern of the OGTT curves of subjects of NGR group were found to be similar without much variations in the blood glucose values within different time points (Fig:3).

We stratified the subjects into three groups by cluster analysis (Table:9 & Fig:6), polynomial (third order) fit (Fig:3 & 4) and area under curve (glucose) calculations (Table:10) to identify and study the subjects with altered glucose metabolism.

OGTT represents whole body glucose tolerance & insulin sensitivity while AUC glucose represents glucose that comes from HGP & unused glucose. The subjects were clustered into three

groups on the basis of all the metabolic variables by a computer program which permits direct visualization of the three dimensional shape of the data set and employed a cluster analysis technique.

Few subjects were found to have altered glucose metabolism (IGR Group), possibly unrecognized diabetes. Almost all of the subjects in the IGR group had a family history of diabetes. Based on current WHO/ADA criteria, interpretation all cases would be considered as being normal. Since neither the current WHO nor ADA criteria make allowance for what happens to blood sugar at one hour, the glucose profile of the subjects in the cluster 1 (IGR Group) would be considered as being entirely "normal" in spite of having abnormally shaped GTT curves.

The cutoff points of AUCg and 1-h PG concentrations for the three groups were then identified by reading from the figures and the corresponding tables (Table:8&10, Fig:7&8). The values are comparable with the mean AUCg values obtained in the cluster analysis of NGR, IGR & all subjects groups (Table:10, 11,12 &13, Fig:8,12 &13).

The correlation between AUCg and 1-h PG concentrations were found to be high mainly in the impaired glucose regulation group making it useful for differentiating it from the NGR group (Fig:9,10&11).

We tried to demonstrate the significance of the cutoff value at 60min time point and the area under the curve of glucose (AUCg) during OGTT for discrimination of various degrees of glucose tolerance (Fig:22, 23&24). We found 1-hr GTT glucose concentration contributed in above studies more than the values of other time points (Table: 10&11, Fig:8). Inclusion of '30 min OGTT insulin' values in the clustering algorithm improves the output marginally. So, we can conclude that the '60 min OGTT glucose' values alone are sufficient to stratify the data and to explore the altered glucose metabolism (Fig:18 and Table:18&19). We found a negative linear correlations between Insulin 30min with Glucose 30min and Glucose 60min (Fig:19 &20).

Several indices of ß-cell responsivity and insulin sensitivity were calculated in order to understand the altered glucose metabolism for comparison the metabolic profiles of groups (Table:20&21, Fig 22). We attempted to explore the hyperbolic relationship between insulin release and ß-cell responsivity (Fig:21).

A summary table is presented to compare different cluster analysis routines with various metabolic variables associated with impaired glucose regulation (Table: 22 & 23).

SUMMARY AND CONCLUSION

The new term ‘pre-diabetes’ or impaired glucose regulation (IGR) was introduced recently and refers to subjects with high fasting plasma glucose (FPG) concentration and normal response to a glucose load (IFG), subjects with abnormal postprandial glucose excursion but normal FPG concentration (IGT), and combination of IGT and IFG.

Research on insulin - glucose dynamics is gaining importance particularly in the area of diabetes prevention. Generally fasting & 2-hour postprandial glucose concentrations are considered as reference points for the diagnosis of diabetes & pre-diabetes. American Diabetes Associations (ADA) guidelines rely on measurement of fasting glucose (7.0 mmol/L) and the European & the WHO groups rely on glucose concentration at 2 hours of OGTT (11.1 mmol/L) in the screening and classification of glucose tolerance. However one-hour glucose concentration is considered for screening and diagnosis of gestational diabetes in the US. Although fasting plasma glucose (FPG) alone does not always detect people with impaired glucose tolerance (IGT) and the 2-h plasma glucose (PG) does not always identify people with impaired fasting glucose (IFG), both tests are useful in terms of their ability to detect hyperglycemia and the consequences of disordered glucose metabolism.

Though both IFG and IGT raise risks for diabetes, some controversies still exist as to the relative contribution of insulin

resistance and beta-cell dysfunction in the progression from impaired glucose regulation (IGR) to diabetes. Insulin resistance and impaired insulin secretion concur toward glucose intolerance and diabetes, but it is unclear which defect arises first, which relates more closely to IFG or IGT, and which reflects different alteration in glucose homeostasis. Some reports showed that subjects with IFG had hyperinsulinemia and/or worsening of insulin resistance, and those with IGT had defective secretion in response to glucose loading, while other reports demonstrated a pronounced defect in early insulin secretion in IFG and marked insulin resistance in IGT.

Insulin resistance is characterized by a decreased ability of insulin to stimulate the use of glucose by the muscle and adipose tissue, where the suppression of lipase controlled by insulin is impaired. The consequent excessive supply of FFAs further affects glucose transportation in the skeletal muscles, and inhibits insulin activity. In the liver, insulin resistance leads to increased HGP, initially compensated by increased insulin secretion. If the process persists, glucotoxicity may occur, leading to chronic hyperglycemia and clinical diabetes.

The OGTT has traditionally been used to classify the status of glucose tolerance for diagnostic purposes: normal glucose tolerance (NGT), IGT and diabetes based on the 2-h PG concentration. The ADA lowered the threshold for IFG from 6.1 mmol / L to 5.6 6.1 mmol / L in order to detect more subjects with pre-diabetes.

We carried out oral glucose tolerance test (OGTT-glucose / insulin) with 29 healthy female individuals for exploring parameters associated with impaired glucose regulation. We observed differences in the shape of their OGTT curves. Variations in glucose concentrations at time points 30 min, 60 min (maximum) and 90 min were observed. The fasting and 2-hr glucose concentrations for all the subjects did not differ significantly. We stratified the subjects into three groups by cluster analysis, polynomial (third order) fit and area under curve (glucose) calculations to identify and study the subjects with altered glucose metabolism. We found 1-hr GTT glucose concentration contributed in above studies more than the values of other time points.

Few subjects were found to have altered glucose metabolism (IGR Group), possibly unrecognized diabetes. Almost all of the subjects in the IGR group had a family history of diabetes. Based on current WHO/ADA criteria, interpretation all cases would be considered as being normal. Since neither the current WHO nor ADA criteria make allowance for what happens to blood sugar at one hour, the glucose profile of the subjects in the cluster 1 (IGR Group) would be considered as being entirely "normal" in spite of having abnormally shaped GTT curves. We calculated several indices of ß-cell responsivity and insulin sensitivity in order to understand the altered glucose metabolism by comparing the metabolic profiles between groups. We attempted to explore the hyperbolic relationship between insulin release and ß-cell responsivity.

We tried to demonstrate the significance of the cutoff value at 60min time point and the area under the curve of glucose (AUCg) during OGTT for discrimination of various degrees of glucose tolerance. The correlation between AUCg and 1-h PG concentrations were found to be high mainly in the impaired glucose regulation group making it useful. It can also be used for identifying individuals with subtypes of pre-diabetes to tailor personalized treatments. One hour OGTT glucose concentrations along with the values of insulin sensitivity indices may help us to understand gestational diabetes mellitus, PCOS and patients of undiagnosed diabetes mellitus with AMI (acute myocardial infarction). Recently, 1-h hyperglycemia (1hPG) during an oral glucose tolerance test (OGTT) with a cut point of 155 mg/dl has been indicated as a further risk factor for type2 diabetes and showed early carotid atherosclerosis. The Honolulu series of studies, showed the one-hour plasma glucose concentration to be an independent risk factor for ischemic heart disease, stroke, and sudden death. They also reported that the vascular risk attached to the one-hour glucose concentration closely followed a gradient pattern with, a direct dose-response relationship.

A clearer understanding of the pathophysiologic abnormalities which characterize IGT and IFG provides insights about interventions to slow/halt the progression to type2 diabetes.

- Subjects with IFG, who manifest predominant liver insulin resistance, are most likely to benefit from agents, e.g., metformin, that reduce hepatic insulin resistance.
- Subjects with IGT, who predominantly have muscle insulin resistance plus severely impaired insulin secretion, are more likely to respond to agents that improve skeletal muscle insulin resistance, such as PPAR- gamma agonists, in combination with an insulin secretagogue, such as GLP-1 analog.
- Both IFG and IGT are characterized by a reduction in early-phase insulin secretion, while subjects with IGT also have impaired late-phase insulin secretion.

We conclude that although the number of subjects studied might be considered relatively small and the data preliminary, the elevated plasma glucose at one hour in the presence of an otherwise non-diabetic OGTT profile can be used as a diagnostic point in the detection and classification of glucose intolerance & as a predictor of long term macrovascular complications. Further study on subjects with elevated one-hour glucose concentration is to be done to determine the natural history of the abnormality in relation to the development of diabetic complications. The acute postprandial hyperglycemia at one hour might in itself be a risk factor.

Timely effective interventions / measures and screening tests for complications at the time of diagnosis become imperative not only for early detection, but also to prevent progression to end stage disease. Life style changes/interventions utilizing low glycemic, high fibre carbohydrates may be useful in preventing and treating all diseases of insulin resistance. Metformin has been shown to lower the risk of myocardial infarction and all-cause mortality by more than 30% in patients with type2 diabetes and obesity, as well as having a beneficial effect on the lipid profile. Drugs such as ß blockers and high dose thiazides exacerbate insulin resistance unlike ACE inhibitors and β- blockers.

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